A STUDY OF THE CONCENTRATION OF HISTAMINE IN THE HYPOPHYSIS AND BRAIN OF THE RABBIT AND OF THE CHANGES PRODUCED BY TREATMENT WITH AMINO ACIDS AND DRUGS

By

Yousif Z. Abou

Thesis presented for the Degree of Doctor of Philosophy, Faculty of Medicine, University of Edinburgh

1968

ACKNOWLEDGEMENTS

The author is indebted to the Faculty of Medicine, University of Edinburgh, for the Graduate Research Fellowship, which made this work possible. I would like to thank my brothers, Edward, George and Ramzi for their kind financial support.

I wish to extend my gratitude to Professor W.L.M. Perry and the members of the teaching staff for their hospitality and assistance. My gratitude and appreciation to Dr H.M. Adam for his constructive criticisms and useful suggestions during the progress of the work and editing of the manuscript of this thesis.

I wish to thank Mr I. Dow and the technical staff for their co-operation and in particular Mr W.R.G. Stephen, for his excellent technical assistance and selfless dedication to the work. I am also grateful for assistance received from Miss Nixon of the Department of Therapeutics.

DEDICATION

TO MY WIFE

for her help, encouragement and understanding;

and to my children, Pat, Paul and Fiona

CONTENTS

List of abbreviations

ATP	Adenosine triphosphate
GABA	gamma-Aminobutyric acid
CNS	Central nervous system
CPZ	Chlorpromazine
'r'	Correlation coefficient
DC	Decarboxylase enzyme
DAO	Diamine oxidase
DOPA	3,4-Dihydroxyphenylalanine acid
DNFB	Dinitrofluorobenzene
EEG	Electroencephalogram
5-HIAA	5-Hydroxyindoleacetic acid
NSD comp.	Hydroxyphenylalkylhydrazines
5-HT	5-Hydroxytryptamine
5-HTP	5-Hydroxytryptophan
ImAA	Imidazoleacetic acid
INMT	Imidazole-N-methyl transferase
i.v.	Intravenous
IPN	Iproniazid
α-MD	α-Methyldopa
MH	1,4-Methylhistamine
MeImAA	1,4-Methylimidazoleacetic acid
MAO	Monoamine oxidase
OPT	ortho-Phthalaldehyde
TCA	Trichloracetic acid

PART I

THE CONCENTRATION OF HISTAMINE IN THE BRAIN, HYPOPHYSIS AND BLOOD OF THE RABBIT

SECTION 1

REVIEW OF PAST LITERATURE

1/1 HISTAMINE IN BRAIN

The study of histamine in the nervous system began when Lewis and Grant (1924) observed the effect of a minute quantity of the amine pricked into the skin. The response was in every way identical with the triple response of local injury. On the basis of this analogy, Lewis (1927) proposed that the response was due to the release in the skin of a substance similar to histamine. The flare seen in the second stage of the response was attributed to an axon reflex which occurred through the action of histamine on sensory nerves in the skin. The disappearance of the flare after nerve degeneration and the extraction from the skin of a substance with the pharmacological properties of histamine, provided the main evidence for this hypothesis (Lewis and Grant, 1924).

These observations raised the question whether or not sensory nerves contained histamine which could be released on stimulation. The presence of histamine in the nervous system of mammals was first demonstrated by Kwiatkowski (1943). He found that most of the histamine resided in the peripheral nerves and that

Table 1

Regional distribution of histamine in brain. Estimated concentrations (ng/g) as reported by various authors.

	Whole brain	Brain-stem	Hypothalamus	Thalamus	Midbrain	Pons-medulla	Cerebral cortex	Cerebellum	Caudate nucleus
Man			2500 (17)	400 (17)	250 (17)	900 (17)	200-400* (17) 100 (22)	500 (17)	500 (17)
Dog	9800-17300 (2) 300 (7) 200-400 (9)		200-6200 (5) 340-900* (13) 215 (15)	<300 (5) 140-270* (13)	200-220* (13)	<30-60* (13)	70 (13) 100-160 (1)	<200 (5) <50 (13) 1500 (1)	<200 (5) 140 (13)
Cat	<30-<60 (5)		430-1150* (19) 700-8300 (5) 1250 (17) 930±110 (23)	75-370* (19) <400 (5) 500 (17) 340±110 (23)	100-160* (19) 280 (1) 600 (17)	<30-110* (19) 100 (1) 400-500* (17)	90 (19) 200 (1) 300-400* (17)	<20-<30 (19) 1500-2000 (1) 200 (17) <50 (5)	50 (19) <600 (5) 500 (17) 340 ± 50 (23)
Rabbit	600 (5) 600 (7) 200-400 (9) 1000 (11) 65 (21)	300-350 (9)	100-200 (22)		300-350 (9) 70±17 (20)		44-50 (1) 200-250 (9) 120±15 (20)	190-240 (9) 64±13 (20)	
Guinea pig	<100† (5) 200-400† (6) 200-300 (9) 1000 (11) 57 (21) 60-90 (24) 73±14 (25)		400 (24) 100-200 (22)	80 (24)	60° (24)	40° (24)	60° (24) 50 (1)	40° (24)	
Rat	4300±1000 (8) 200-400 (9) 200-400 (12) 220-310 (16) 178±40 (15) 53±5 (14) 52±1 (18) 38±3 (20) 76 (21) 60±10 (22) 60-90 (24)	50 ± 10(18)	4300±300 (10) 170±20 (22) 560 (24)	110 (24)	50 (24)	30 (24)	28±4 (18) 50 (24)	38±3 (18) <20-24 (24)	

Table to be continued.

Table 1 continued

	Whole brain	Brain-stem	Hypothalamus	Thalamus	Midbrain	Pons-medulla	Cerebral cortex	Cerebellum	Caudate nucleus
Cattle	100-700† (4)	1000† (4)					500 (22)	2000-3000† (3)	20 (5)
Frog	350±30 (22)		760 (22)	310 (22) (optic lobes)			290 (22)	60 (22)	
Chick (1-21 days old)	120 (24)				70, 90 (24)	90, 110 (24)	50, 110 (24)	110, 200 (24)	
Pigeon	90, 110 (24)								
Mouse	70 (24)								

( ) reference

x range of concentrations in different parts

† values given in terms of histamine-dihydrochloride

• One value.

All the above results were obtained by biological assay except in ref. nos. 8, 9, 11, 12, 15, 17, 18, 21 and 23 which were assayed by chemical methods. Results of (22) were assayed by isotopic-enzymatic procedure.

References

(1) Kwiatkowski, 1943
(2) Cicardo and Stoppani, 1949.
(3) Werle and Weicken, 1949.
(4) Euler, 1950.
(5) Harris, Jacobsohn and Kahlson, 1952.
(6) Strengers and Maas, 1956.
(7) West, 1957.
(8) Clouet, Gaitonde and Richter, 1957.
(9) Shore, Burkhalter and Cohn, 1959.
(10) Szeberényi and Kovács, 1959.
(11) Waalkes, Coburn and Terry, 1959.
(12) Burkhalter, Cohn and Shore, 1960.
(13) Adam, 1961.
(14) Carlini and Green, 1963.
(15) Ungar and Witten, 1963.
(16) Moran and Westerholm, 1963.
(17) McGeer, 1964.
(18) Green and Erickson, 1964.
(19) Adam and Nye, 1966.
(20) Crossland, Woodruff and Woodruff, 1966.
(21) Kremzner and Pfeiffer, 1966.
(22) Snyder, Baldessarini and Axelrod, 1966.
(23) White, 1966.
(24) Adam, unpublished results.
(25) Michaelson, 1967.

in the central nervous system the quantity was small and often not detectable. Later, Harris, Jacobsohn and Kahlson (1952) reported that brain adjoining the hypophysis was rich in histamine. Thus in the dog, cat and pig, the concentration was high in the hypothalamus, notably in the eminentia media; elsewhere in the brain it was too low to be detected by their method.

Other workers have since reported the presence of histamine in the brain of various species (Table I). Values for the concentration are seen to differ widely depending on the method used to estimate histamine. For example, Shore, Burkhalter and Cohn (1959) employed a sensitive spectrofluorometric method of assay and found in the dog and rabbit that the concentration of histamine was more or less the same in different parts of the brain. The use of refined biological methods, however, led to a different conclusion, namely, that in the dog (Adam, 1961) and cat (Adam and Hye, 1966) histamine was unevenly distributed in the brain. Thus, the concentration was highest in the hypothalamus, intermediate in the thalamus and midbrain and lowest in the pons, medulla and cerebral cortex; none was detected in the cerebellum or white matter. As

pointed out by these authors, the concentration of histamine in different parts of the brain was roughly similar to that reported for noradrenaline (Vogt,1954) and 5-HT (Amin, Crawford and Gaddum, 1954).

1/2 HISTAMINE IN THE HYPOPHYSIS

The isolation and chemical identification of histamine in the hypophysis is due to Abel and Kubota (1919) who extracted it from bovine glands. Estimates of its concentration in different parts of the gland were not reported until much later (Harris et al,1952). The high values encountered in the stalk led to a reinvestigation of the occurrence of histamine in brain. In the species studied, the values obtained for the hypophysis were high and varied within wide limits (Table 2). These findings were later confirmed and extended for the dog (Adam, 1961) and cat (Adam and Hye, 1966), where it was also shown that in the stalk and posterior lobe a proportion of histamine derives from mast cells. It was conceivable, therefore, that the variation in histamine concentration might depend on the number of mast cells present in the gland.

Table 2

Estimates of histamine concentration in the hypophysis (µg/g) as reported by various authors

Species	Anterior lobe	Posterior lobe	Hypophysial stalk	Reference
Dog	1.0-19:0 2.3-15.2	0.4-26.5 2.0-23.0	5.7-30.0 8.0-27.0	Harris et al, 1952 Adam, 1961
Cat	0.26-43.0 0.84-6.53	<0.1-76.0 0.56-3.45	3.6-25.5 1.2-9.3	Harris et al, 1952 Adam and Hye, 1966
Pig	0.1 - 1.0	1.2 - 7.0	2.3-11.0	Harris et al, 1952
Guinea pig	Whole hypophysis 0.7 - 1.27			Abou, Adam and Stephen, unpublished results
Rat	0.17 - 0.61			" "

1/3 MAST CELLS IN THE NERVOUS SYSTEM AND HYPOPHYSIS

Peripheral nerves. Mast cells are common in many tissues of higher vertebrates and are a rich source of histamine. Their presence in the peripheral nerves and apparent absence in the CNS may explain the difference in concentration of histamine in these two parts of the nervous system. It is still not known how much histamine in peripheral nerves derives from mast cells (Gamble and Goldby,1961; Gamble, 1964; Ashhurst and Richards,1964). In the rabbit and cattle, only part of the histamine extractable from peripheral nerves is in mast cells (Werle and Schauer, 1956). These workers found that although histamine and mast cells are plentiful in the splenic nerve of cattle, the vagus nerve contains much histamine yet few mast cells. Findings on the subcellular localization of histamine in peripheral nerves suggest that the amine also resides in cells other than mast cells. Most of the histamine in mast cells is contained in particles of a higher density than mitochondria (Hagen, Barrnett and Lee, 1959 ; Green and Furano, 1962; Lagunoff and Benditt, 1963), whereas histamine present in homogenate of splenic nerve of cow is found in the supernatant, (Euler, 1958). Moreover, compound 48/80, which

disrupts mast cells, released only a small proportion of histamine in peripheral nerves (Feldberg and Greengaard, 1956). Kwiatkowski (1943) did not observe any consistent change in the histamine content of the sciatic nerve of rat and cat after nerve section. Similar results were reported by Werle and Weicken (1949) and Werle and Palm (1950). Hence, it would seem that histamine is not present in nerve axons.

Brain. Mast cells have not been found in the brain of mammals (Zimmerman, 1908; Gray, 1935; Constantinides, 1953; Riley and West, 1955; Padawer, 1957; Nepryakhin, 1960; Adam, 1961; Kelsall and Lewis, 1964; Adam and Hye, 1966), but have been located in the meninges of rat (Waldeyer, 1875; Wislocki and Leduc, 1952), in dog's choroid plexus (Tsusaki, Eriguchi and Kojo, 1951; Adam, 1961) and in the connective tissue around blood vessels near the lateral and third ventricle in the brain of hamster (Kelsall and Lewis, 1964). The hedgehog seems to be the only mammal to contain mast cells within its brain substance (Krabbe, discussion to Quensel,1928). As in the dog and cat, mast cells have not been detected in the rabbit brain (Constantinides, 1953).

Hypophysis. Mast cells are present in the

hypophysis of dog (Arvy and Quivy, 1951; Adam, 1961) and cat (Gray, 1935; Adam and Hye, 1966). In these two species mast cells were found in all parts of the hypophysis but mostly in the posterior lobe and pars tuberalis of the adenohypophysis, where this forms part of the hypophysial stalk (Adam, 1961; Adam and Hye, 1966). These authors suggested that histamine extractable from the posterior lobe and pars tuberalis probably derives from mast cells. The anterior lobe (pars distalis) differs from the other parts of the gland in that it contains more than half of the histamine extractable from the entire gland, but only a small portion of the mast cells. It is therefore probable that in this part of the gland some of the histamine resides in cells other than mast cells. The results obtained with compound 48/80 are consistent with this view (Adam and Hye, 1966).

1/4 SUBCELLULAR DISTRIBUTION OF HISTAMINE IN BRAIN AND HYPOPHYSIS

Little is known about the subcellular distribution of histamine and its localization in cells of the brain. Since histamine is not associated with mast cells, it must be present in some other type(s) of cell.

In brain of guinea pig, dog (Michaelson and Dow, 1963; Michaelson Coffman, 1967) and rat (Carlini and Green, 1963) the amine is present in particles which are much smaller and less dense than those found in mast cells. Michaelson and Dow (1963) reported that most of the 'bound' histamine in dog's hypothalamus sedimented with crude mitochondrial and microsomal fractions, whereas most of the hypophysial histamine was contained in particles which, like mast cell particles, sedimented in the low speed nuclear fraction. Subfractions of hypothalamus which contained a high proportion of the total histamine were also shown by electron microscopy to contain large numbers of pinched-off nerve endings or synaptosomes. Recent reports confirm these findings (Katoka and De Robertis, 1967): histamine in the rat brain occurs in sub-fractions containing small nerve endings and synaptic vesicles which were identified by electron microscopy.

The subcellular distribution of histamine in brain resembles that of acetylcholine (Hebb and Whittaker, 1958; Whittaker, 1959) and 5-HT (Whittaker, 1959; Michaelson and Whittaker, 1963) and is quite unlike that of histamine in mast cells (Hagen et al, 1959; Green and Furano, 1962). These observations

support the conclusion (Adam, 1961) that histamine in brain is not in mast cells.

1/5 HISTAMINE IN BLOOD

It was generally supposed that most of the histamine in blood appeared in the leucocytes, particularly the eosinophil (Code, 1952). Later Graham, Lowry, Wheelwright, Lenz and Parish (1955) demonstrated that about half of the histamine in normal blood was contained in the basophils. In man, the basophil is the predominant and perhaps the exclusive carrier of histamine in peripheral blood (Valentine, Lawrence, Pearce and Beck, 1955). In the dog and to some extent in the guinea pig, histamine is found mainly in the eosinophil (Code and Mitchell,1957). In the rabbit, however, not only is the blood histamine higher than in other species, but the histamine is carried mainly in the platelets (Minard, 1937; 1941; Zon, Ceder and Crigler, 1939; Humphrey and Jaques, 1954). (Table 3).

Like mast cells (Schayer, 1956a) the rabbit platelets are a rich source of histidine decarboxylase (Schayer and Kobayashi, 1956). When the platelets

Table 3

Estimates of concentration of histamine in the rabbit blood (µg/ml) as reported by various authors

Whole blood

9.6 - 12.0	(Barsoum and Gaddum, 1935)
0.6 - 2.7	(Code, 1937a)
0.2	(Riesser, 1937)
7.3	(Tarras-Wahlberg, 1937)
1.0 - 3.8	(Zon, Ceder and Crigler, 1939)
0.5 - 2.3	(Dragstedt and Rocha e Silva, 1941)
1.8 - 5.0	(Minard, 1941)
0.9 - 8.6	(Wilson, 1941)
2.4 - 3.8	(Rocha e Silva and Essex, 1942)
0.07 - 2.8	(Rocha e Silva, Graña and Porto, 1945)
0.4 - 3.2	(Graña and Rocha e Silva, 1945)
2.5 - 4.5	(Emmelin, 1945)
2.2 - 3.5	(Code, Dews and Higgins, 1953)
1.2 - 2.9	(Weissbach, Waalkes and Udenfriend, 1958)
1.8 - 4.9	(Shore, Burkhalter and Cohn, 1959)
1.6 - 4.0	(Waalkes, Coburn and Terry, 1959)

Plasma

0.02 - 0.056	(Code, 1937b)
0.002	(Humphrey and Jaques, 1954)

are incubated with ^{14}C-histidine, they form histamine and bind it in stable condition. The platelet, enzyme shares the properties of histidine decarboxylase and aromatic L-amino acid decarboxylase (Schayer, 1966). Like the decarboxylase from rabbit and guinea pig kidney, it combines firmly with the coenzyme, pyridoxal phosphate (Schayer, 1959); optimum activity is in the range pH 7.2 - 7.4, or midway between the pH optima of the two enzymes. The enzyme appears to be specific for histidine, since rabbit platelets do not synthesize 5-HT (Gaddum and Giarman, 1956; Udenfriend, 1958). The concentration of 5-HT in rabbit blood is similar to that found for histamine (Table 4).

1/6 HISTAMINE FORMATION IN BRAIN AND HYPOPHYSIS

Mammalian decarboxylase (DC) for histidine was first demonstrated by Werle (1936), Holtz and Heise (1937) and Werle and Herrmann (1937). The highest activity was found in the rabbit and guinea pig kidney. Further evidence was obtained by Schayer (1952) and Waton (1956).

Decarboxylases for histidine. Animal tissues

Table 4

Estimates of concentration of 5-HT in the rabbit blood (µg/ml) as reported by various authors.

Whole blood	
3.5	(Shore, Pletscher, Tomich, Kuntzman and Brodie, 1956)
4.0 - 6.0	(Weissbach, Waalkes and Udenfriend, 1957, 1958)
3.2 - 6.0	(Waalkes and Coburn, 1959)
3.3 - 6.0	(Waalkes, Coburn and Terry, 1959)
Serum	
1.8 - 5.6	(Erspamer, 1954)
3.3 - 10.0	(Davis, Meeker and Bailey, 1961)
Platelet-free plasma	
0.01 - 0.07	(Kärki, Paasonen and Peltola, 1960)
0.05 - 0.10	(Waalkes and Coburn, 1959)
0.002	(Humphrey and Jaques, 1954)

contain at least two enzymes which can decarboxylate histidine. The relative importance, distribution and role of these enzymes in the formation of histamine is not yet clear. Properties attributed to the enzymes are summarized in Table 5.

(a) Mast cells, rat stomach, rat hepatoma and certain rapidly-growing tissues contain decarboxylase which has a high affinity, and is specific for L-histidine.

(b) The DC from rabbit and guinea pig kidney has low affinity for L-histidine, as compared with L-5-HTP and L-DOPA. This 'non-specific' DC has been designated by Lovenberg, Weissbach and Udenfriend (1962) as 'aromatic L-amino acid decarboxylase'. It is still not clear whether this DC consists of one or more enzymes. There is evidence to suggest that decarboxylation of several aromatic amino acids, including histidine, is by a single enzyme (Westermann, Blazer and Knell, 1958; Yuwiler, Geller and Eduson, 1959; Rosengren, 1960; Ganrot, Rosengren and Rosengren, 1961; Lovenberg et al, 1962). But Clark, Weissbach and Udenfriend (1954) claimed to have separated the 5-HTP DC from DOPA DC; and Werle and Aures (1959) reported the separation of a DC for histidine from DOPA DC.

Table 5

Properties of two decarboxylases for histidine

Characteristics	Histidine decarboxylase from mouse mastocytoma	Aromatic L-amino acid decarboxylase from guinea pig kidney
pH optimum	6.0 (6)	9.0-9.5 (6) 9.0 (7)
K_m	5×10^{-4}M(6)	5×10^{-2}M (6,7)
Effect of dialysis on activity	marked loss (rat peritoneal mast cells (3)	insignificant loss (rabbit kidney) (1,2)
Substrate specificity	histidine (6)	histidine and other aromatic amino acids (4,6,9)
Effect on activity of:		
(a) Benzene	none (6)	activation (6,7,9)
(b) α-Methyldopa (10^{-3}M)	none (6)	strong inhibition (5,6,8)
(c) α-Methylhistidine	15×10^{-4}M* (10)	150×10^{-4}M (10)
(d) α-Hydrazino analogue of histidine	7×10^{-5}M† (11)	80×10^{-5}M (11)
(e) 4-Bromo-3-hydroxybenzyloxyamine (NSD-1055)	0.0027×10^{-4}M† (10) 0.04×10^{-5}M* (11)	0.0014×10^{-4}M (10 0.009×10^{-5}M (11)

( ) reference
* concentration required for 50% inhibition
† concentration required for 75% inhibition

References

(1) Werle and Herrmann, 1937
(2) Werle and Krautzun, 1938
(3) Rothschild and Schayer, 1959
(4) Werle and Aures, 1959
(5) Mackay and Shepherd, 1960
(6) Weissbach, Lovenberg and Udenfriend, 1961
(7) Mackay, Riley and Shepherd, 1961
(8) Werle, 1961
(9) Lovenberg, Weissbach and Udenfriend, 1962
(10) Reid and Shepherd, 1963
(11) Levine, Sato and Sjoerdsma, 1965.

Studies on brain.

(a) In vitro: Brain of cattle (Holtz and Westermann, 1956), rat (Schayer, 1956b; Kahlson, Rosengren, Westling and White, 1958; Lovenberg et al, 1962), pig and cat (White, 1959) has been shown to decarboxylate histidine. DC activity was highest in the hypothalamus and least in the cerebral cortex and cerebellum; the newly-formed histamine appeared to be loosely 'bound', since the greater part was found in the supernatant after centrifugation (White, 1959). Adam, Hye and Waton (1964) have confirmed the presence of DC for histidine in cat hypothalamus. Although DC enzyme from various tissues has been extensively studied, little is known about the DC enzyme of brain. The pH of the incubation medium used in some in vitro studies was 7.4 (Kahlson et al, 1958; Schayer, 1962), which is midway between the pH optima of the two enzymes shown in Table 5. Lovenberg et al (1962) studied the substrate specificity of a partially purified DC enzyme from dog brain stem. They found that several amino acids, including histidine, were decarboxylated by this enzyme preparation at pH 9.0. The relative rates of activity towards the various amino acids were similar to those obtained with rabbit

kidney enzyme. Although these authors did not study the effect of inhibitors on brain DC, they concluded that it was similar to the kidney enzyme.

Little is known about the formation of histamine in the rabbit brain either *in vitro* or *in vivo*. Werle and Krautzun (1938) incubated rabbit brain with histidine but were unable to detect the formation of histamine. Recently, however, it has been shown that when the hypothalamus from cat or rabbit is incubated with ^{14}C-histidine, histamine formation takes place at pH 8.0 but not at pH 6.0 (Adam, Hye and Waton, unpublished results).

(b) *In vivo*: Formation of ^{14}C-histamine in cat brain has also been demonstrated after intraventricular administration of ^{14}C-histidine (White, 1960; Jonson and White, 1964). Since histamine is not known to enter from blood into the brain (Halpern, Neveu and Wilson, 1959; Adam et al, 1964; Snyder, Axelrod and Bauer, 1964; Snyder and Axelrod, 1965), it can be concluded that histamine contained in brain is formed locally. "This assumption would imply that imidazole-N-methyl transferase in brain, together with histidine decarboxylase is concerned with the physiological

regulation of local histamine turnover" (Kahlson and Rosengren, 1965).

Studies on hypophysis

In the dog and cat some of the hypophysial histamine is presumed to derive from mast cells, since these cells were shown to be capable of decarboxylating histidine (Schayer, 1956a; Hagen, Weiner, Ono and Lee, 1960; Weissbach et al, 1961; Day and Green, 1962). Adam et al (1964) were unable to detect DC activity in cat's hypophysis when incubated with ^{14}C-histidine for 3 hr. Again, after intravenous infusion of ^{14}C-histidine in the cat, ^{14}C-histamine was detectable in the brain but not in the hypophysis. These findings are in agreement with the slow turnover rate of histidine to histamine in mast cells (Schayer, 1952).

1/7 CATABOLISM OF HISTAMINE IN BRAIN AND HYPOPHYSIS

Diamine oxidase

Best and McHenry (1930) were the first to detect the enzyme histaminase in mammalian tissues; they regarded the enzyme as being specific for histamine. Later, Zeller (1938) reported that animal tissue preparations were able to destroy by oxidative

deamination not only histamine but also diamines; he therefore substituted the term 'diamine oxidase' (DAO) for histaminase. On the basis of *in vitro* studies histaminase is identified by some workers with DAO (Tabor, 1954; Zeller, 1938; Zeller, 1965), while others maintain that there are two distinct enzymes (Kapeller-Adler and Renwick, 1956; Blaschko, Friedman and Nilsson, 1958; Kolb, 1956). The subject has been frequently reviewed (for recent reviews, see Zeller, 1965; Buffoni, 1966). In both cases the reaction product is the same. In this review the two terms are used interchangeably.

The main product of oxidation by DAO is imidazole acetic acid (ImAA) (Tabor, 1951). ImAA is also excreted in the form of conjugate with ribose when large doses of histamine or ImAA are given to rats (Karjala, 1955; Tabor and Hayaishi, 1955).

The brain displays very low DAO activity, if any (Zeller, Birkhäuser, Mislin and Wenk, 1939; Birkhäuser, 1940; Cotzias and Dole, 1952). Cotzias and Dole (1952) reported the presence of low DAO activity in the brain of rat and mouse, but the activity was not detected in rabbit and guinea pig brain. These findings were later confirmed by Burkard, Gey and

Pletscher (1963) who incubated ^{14}C-putrescine and cadaverine with brain homogenate from four rodent species; degradation of diamines could not be detected. When ^{14}C-histamine was injected into the perfused cerebral ventricles of the cat, ImAA was not detected in the perfusate (White, 1960).

Methylating enzyme

Originally it was believed that DAO was the only enzyme responsible for the catabolisms of histamine (Zeller, 1951; Tabor, 1954). However, it was later shown that a major pathway for histamine catabolism in mammals is by methylation in the imidazole ring to yield I-methyl-4(β-aminoethyl) imidazole (I,4-methylhistamine; MH) (Schayer,1956b; Schayer and Karjala, 1956; Schayer, 1959; Brown, Tomchick and Axelrod, 1959).

Properties of the methylating enzyme (imidazole-N-methyl transferase; INMT) have been studied *in vitro* (Lindahl, 1958a; 1958b, 1958c, 1960; Brown et al,1959). In both liver and brain, the enzyme activity is in the soluble cytoplasm (Brown et al, 1959; Lindahl, 1960) and appears to be specific for histamine. The methylation of histamine requires S-adenosyl-methionine (Brown, Axelrod and Tomchick, 1959; Lindahl,1960),

which is present in brain (Baldessarini and Kopin, 1963). MH has been shown to inhibit methylation of histamine *in vitro* (Brown et al, 1959; Lindahl,1960).

Formation of MH and its oxidative product, 1,4-methylimidazole acetic acid (MeImAA) has been demonstrated in cat brain by the use of isotopic methods, both *in vitro* (White, 1959) and *in vivo* (White, 1960; Jonson and White, 1964). Mammalian brain is rich in methylating enzyme (INMT), and the highest activity was found in the guinea pig brain (Brown et al, 1959). The regional distribution of INMT has been studied in the brain of monkey: the activity was highest in the hypothalamus and hypophysis (Axelrod, MacLean, Albers and Weissbach, 1961). In rabbit brain INMT has been found in the brain stem, midbrain, cerebellum and cerebral cortex. (Brown et al, 1959).

1,4-Methylhistamine. This was synthesized (Pyman, 1911) and its activity on isolated organs described (Lee and Jones, 1949) before its isolation from mammalian tissues. ^{14}C-MH appears in urine of rabbit and other species after the administration of ^{14}C-histamine (Schayer, 1956b). MH has been detected in brain of guinea pig, rat, rabbit and monkey (Fram

and Green, 1963) and cat (Perry, Hansen, Foulks and Ling, 1965; White, 1966), but not in the cerebrospinal fluid (Perry, Hansen and Jenkins, 1964). The concentration of MH in guinea pig brain was found to be in the range of 45-75 ng/g (Fram and Green, 1965). In the thalamus, caudate nucleus and mesencephalon of the cat, the concentration of both histamine and MH were approximately equal; the mean values ranged from 270 ng/g in the mesencephalon to 560 ng/g in hypothalamus (White, 1966).

Although histamine has a low affinity for monoamine oxidase (MAO) (Zeller, Stern and Blanksma, 1956; Lindell and Westling, 1957), MH is catabolized by this, or a closely related, enzyme (Schayer and Kobayashi, 1956; Lindell and Westling, 1957; Rothschild and Schayer, 1958; Davison, 1958). Inhibition of MAO prevents the formation of MeImAA in brain (White, 1960) and other tissues (Kobayashi and Schayer, 1956; Lindahl, 1958a; Lindell, Nilsson, Roos and Westling, 1960). When the cerebral ventricles of the cat were perfused with ^{14}C-histamine, MH and MeImAA appeared in the perfusate (White, 1960); it was also observed that histamine introduced in this way was methylated to a greater extent than histamine which was formed

from histidine administered in the same manner.

The question arises whether methylation in the ring converts histamine into a substance which acts on specific receptors in the brain or whether it represents only the first step in the inactivation of histamine. Not much is known about the effects of MH on the brain (S.I/II) or/its localization in the cell.

Monoamine oxidase

In rat brain the major metabolite of histamine was ImAA and only small amounts of MH were formed after the intraventricular injection of ^{3}H-histamine (Snyder, Glowinski and Axelrod, 1966). This finding agrees with the relatively low activity of INMT in rat brain (Brown et al, 1959). Since inhibition of MAO retarded the disappearance of labelled histamine in rat brain, it might be argued that histamine was metabolized predominantly by MAO (Snyder et al,1966).

Other routes of histamine metabolism

Other histamine catabolites have been reported in the mammalian brain. Robinson and Green (1965) found ImAA riboside and ImAA ribotide in the rat brain after the injection of ^{14}C-histidine. Snyder

et al (1966) obtained no evidence for the formation of these catabolites after the intraventricular injection of ^{3}H-histamine.

Certain enzymes catalyse the interaction of histamine either with diphosphopyridine nucleotide (Alivisatos, 1958) or with triphosphopyridine nucleotide (Abdel-Latif and Alivisatos, 1961), thus leading to the formation of di- and triphosphohistamine nucleotide. These compounds were not detected in rat brain after the administration of ^{14}C-histidine (Robinson and Green, 1965).

1/8 UPTAKE OF HISTAMINE BY BRAIN AND HYPOPHYSIS

In vitro. Subcellular fractions of rat brain which contain histamine also take up the amine. The uptake of histamine in vitro by nerve ending particles and microsomes was similar to that of 5-HT; as were the concentration ratios of 5-HT and histamine between particles and media (Robinson, Anderson and Green, 1965). ATP and magnesium ions failed to stimulate uptake of 5-HT by nerve ending particles, which suggests that the uptake was not an active metabolic process. The uptake of histamine

by brain fractions was considered to be non-specific under the conditions of the experiments (Robinson et al, 1965). Brain slices take up L-histidine more efficiently than do other tissues and in preference to several other amino acids (Neame, 1961; 1962). Brain slices also take up histamine, but to a lesser extent than histidine (Neame, 1964).

In vivo. ^{14}C-histamine injected in the cerebral ventricles of the cat is taken up by brain and catabolized (White, 1960). After the injection of 500µg of histamine base intraventricularly, some of the amine appeared in the blood stream (Bhawe, 1958). When the cerebral ventricles were perfused with a solution containing histamine (for 1 hr), some diffused into the brain and more was found in the grey matter than in white matter (Draskosi, Feldberg, Fleischhauer and Haranath, 1960). When ^{3}H-histamine was administered intraventricularly in the rat, it was taken up and retained in the brain, from which it disappeared at first rapidly (half-life 1.6 hr from 1 to 6 hr) and then more slowly (half-life 11 hr from 6 to 24hr)(Snyder, Glowinski and Axelrod, 1966). This is in marked contrast to the fate of exogenous

histamine in peripheral tissues (Snyder, Axelrod and Bauer, 1964). The retention of radioactive histamine in the brain suggests that it could be bound in a fashion which protects it from rapid enzymatic destruction (Snyder et al, 1966). Labelled histamine in brain was associated with the "nerve-ending" and the 'microsome myelin' fractions. Very little amine was found in the mitochondrial fraction or in nuclei and débris. In contrast, ^{3}H-ImAA was essentially confined to the supernatant. But when unlabelled brain was homogenized with radioactive histamine and then fractionated, almost all radioactivity was recovered in the supernatant (Snyder et al, 1966).

After the intravenous infusion of ^{14}C-histamine in the cat, some was found in the hypophysis, especially in the anterior lobe, but none was detected in the hypothalamus or area postrema (Adam et al,1964; S. 1/6). Thus it appears that the hypophysis is able to take up exogenous histamine but is unable to form it from isotopic histidine; in contrast, the hypothalamus is unable to take up histamine from blood but is able to decarboxylate histidine. In the rat, Halpern Neveu and Wilson (1959) reported that labelled histamine

penetrated into all the tissues examined except the brain. However, other workers (Robinson and Green, 1964; Snyder, Axelrod and Bauer, 1964; Snyder and Axelrod, 1965) found that minute quantities of histamine are taken up from blood by the rat brain, but that none penetrated the mouse brain (Snyder et al, 1964; Snyder and Axelrod, 1965).

1/9 FATE OF HISTAMINE IN THE RABBIT

It has long been known that rabbits can tolerate the infusion of large doses of histamine; since only a small amount was found in the urine, it was presumed that most of the histamine was destroyed (Oehme, 1913; Guggenheim and Löeffler, 1916). In 1929, Best showed that many tissues of the body were capable of inactivating histamine in vitro. Later it was found in various species that histamine disappeared rapidly from blood (Dragstedt and Mead, 1935; Rose and Browne, 1938; Alexander, 1946; Emmelin, 1951). Little is known about the distribution of histamine in rabbit tissues. Minutes after the intravenous injection of the amine in the rat, the greatest concentrations were found in the kidney,

heart, liver and lung (Halpern et al, 1959), a distribution that appears to reflect, in part, the blood flow (Green, 1967). The persistence of histamine in the spleen may be partly due to its content of platelets; platelets from man (Weissbach and Redfield, 1960), dog (Weissbach, Bogdanski and Udenfriend, 1958) and rabbit (Markwardt, Barthel and Glusa, 1966) have been observed to take up histamine in vitro. Histamine seems to enter the platelet membrane by passive diffusion. This process was not inhibited by local anaesthetics (Markwardt et al, 1966). Most of the histamine taken up by tissue is catabolized (Schayer, 1966). The cells responsible for the uptake are not known. However, histamine can be taken up by red cells and the white cell fraction of human, dog and rabbit blood (Anrep, Barsoum, Talaat and Weininger, 1939; Lindell and Viske, 1961).

According to Rose and Weil (1939), the blood histamine of the rabbit fell invariably during anaphylaxis and only seldom did a transient increase in plasma histamine occur. Further, when histamine was injected during the anaphylactic reaction, the

concentration of the amine did not rise in the whole blood or in the plasma; it was therefore concluded that a mechanism exists for the removal of large quantities of histamine from plasma (Rose, 1941).

In the rabbit, histamine is metabolized by oxidation and by methylation to approximately the same extent (Schayer, 1956b). After subcutaneous injection of ^{14}C-histamine, Schayer found MeImAA and ImAA riboside as the main catabolites in urine. This was in contrast to the findings in the cat and dog where the main route of metabolism of injected histamine is by methylation.

1/10 METABOLISM OF HISTIDINE

The metabolism of histidine has been reviewed by Tabor (1954). The major pathways of L-histidine utilization are (a) incorporation into proteins, (b) excretion in urine and (c) degradation; the laster is mainly by histidase and amino acid oxidase, and quantitatively is the most important (Tabor, 1954). Within one hr after i.v. injection of isotopic histi--dine in mice, about one third of radioactivity was

found in the expired CO_2 (Borsook, Deasy, Haagen-Smit, Keighley and Lowy, 1950).

Little is known about the fate of histidine in the rabbit. Urocanic acid has been isolated from rabbit's urine after the administration of histidine (Kiyokawa, 1933; Darby and Lewis, 1942); this finding, however, was not confirmed (Edlbacher, 1943, quoted by Tabor, 1954).

Information on metabolism of histidine in brain is also meagre. Values have been reported for the concentration of free histidine in the whole brain of several species (9 to 25 μg/g); imidazole compounds that have so far been identified in mammalian brain are given in Table 6. Robinson and Green (1965) identified ImAA riboside and ribotide in rat brain after parenteral administration of ^{14}C-histidine (S.1/7). Carnosine (β-alanylhistidine) (Abraham, Pisano and Udenfriend, 1961), homocarnosine (gamma-aminobutyrlhistidine) (Pisano, Abraham and Udenfriend, 1963), I- and 3-N-methylhistidine (Tallan, Moore and Stein, 1954) and ergothioneine (betaine of thiol-histidine) (Crossland, Mitchell and Woodruff, 1966)

have been found in mammalian brain (Table 6). In rat brain, Brown and Kies (1959) were unable to detect urocanase activity or the formation of hydantoin propionic acid. When rats were given histidine by i.v. injection (Friedberg and Greenberg, 1947), the amino acid was highly concentrated by liver and kidney, less so by skeletal muscles and not at all by brain; 80 to 90 per cent of histidine was removed from blood plasma within 15 minutes. Despite an apparent barrier to the uptake of histidine by brain, the free amino acid content of brain in untreated rats was found to be 7 times as great as that in plasma. Kamin and Handler (1951) reported small but significant increment in the concentration of histidine in dog brain after continuous i.v. infusion of the amino acid. Decarboxylation of histidine in brain has already been discussed (S.1/6).

1/11 PHYSIOLOGICAL SIGNIFICANCE OF HISTAMINE IN BRAIN

'Histamine in the CNS has been ignored for a long time by several investigators as a second-class amine. But this amine, however annoying the fact may be, has the same citizenship rights in the CNS as

TABLE 6

Imidazole compounds identified in mammalian brain

Compound	Species	Reference
Histamine		Table I.p.
1,4-Methylhistamine	Rat, guinea pig, rabbit, monkey	Fram and Green, 1963.
	guinea pig	Fram and Green, 1965
	cat	Perry et al, 1965
	cat	White, 1966
1,4-Methylimidazole acetic acid *	cat	White, 1960
Histidine	rat	Friedberg and Greenberg, 1947.
	rat	Schurr et al, 1950.
	rat	Williams et al, 1950
	dog	Kamin and Handler, 1951.
	cat	Tallan et al, 1954.
	rat	Clouet et al, 1956.
Imidazole acetic acid *	rat	Snyder et al, 1966.
Imidazole acetic acid riboside and ribotide. †	rat	Robinson and Green, 1965.
1- and 3-Methyl-histidine	cat	Tallan et al, 1954.
Homocarnosine	rat, guinea pig, rabbit, cat, dog, bovine, monkey, man.	Pisano et al, 1963
Carnosine	as for homocarnosine	Abraham et al, 1961
Ergothioneine	mouse, rat, guinea pig, rabbit, cat, sheep.	Crossland et al, 1966

* Identified after the administration of labelled histamine

† Identified after the administration of labelled histidine.

catacholamines and 5-HT, whose function in the CNS is approximately as obscure as that of histamine" (Erspamer, 1961). Virtually nothing is known about the physiological role of histamine in the CNS, though "the uneven distribution of a pharmacologically active substance in the brain strongly suggests that the agent has a role to play in the specialised function of those regions where its concentration is high" (Vogt, 1959). Histamine, like monoamines, is unevenly distributed in brain (Adam, 1961; Adam and Hye, 1966). Parts of the brain which contain histamine also contain MH (White, 1966) and histamine methylating activity. This enzyme, like MAO, is widely distributed in the brain (Axelrod et al,1961). Histamine-forming-capacity in brain (White, 1959) follows roughly the same pattern of distribution as histamine.

The pronounced peripheral effects of histamine and its inability to cross the blood brain barrier make it difficult to study any direct actions which it might have on the CNS (Sokoloff, 1959; Virno, Gertner and Bovet, 1956).

In the whole animal, an apparent narcotic effect

of histamine was observed in the cat (Dale and Laidlaw, 1911). It has been reported to produce 'catatonia' in mice (DeJong, 1945), changes in the EEG pattern in rats (Brandon, 1955; Bovet, Kohn, Marotta and Silvestrini, 1958) and rabbits (Goldstein, Pfeiffer and Monoz, 1963), and to stimulate the vasomotor centre in cats and dogs (Feldberg and Kwiatkowski, 1933; Pilcher and Sollman, 1914). Histamine inhibits transcallosally evoked potentials in the cat after intra-carotid injection (Gilfoil, Hart and Marrazzi, 1960). Similarly in the rabbit it increases the electrical activity of the cerebellum (Crossland and Mitchell, 1956).

The central actions of histamine can be studied by injecting the drug into the cerebral ventricles. In the conscious cat, a dose of 150 - 200 µg caused marked changes in behaviour: sedation, salivation, increased respiration and muscular weakness (Feldberg and Sherwood, 1954); in the anaesthetized cat, a pressor response was observed which was prevented by intraventricular injection of mepyramine (Trendelenburg,1957a; White, 1961b). Trendelenburg (1957b) suggested that histamine stimulated sympathetic

ganglion cells in the brain, since by analogy, small doses of histamine facilitate transmission of impulses through the sympathetic cervical ganglion of the cat. However, Gertner and Kohn (1959) reported that histamine depresses ganglionic transmission.

Further, histamine has been applied to the CNS by electrophoresis through micropipettes and the electrical activity was recorded simultaneously. In such experiments histamine did not affect synaptic transmission in the lateral geniculate body of the cat (Curtis and Davis, 1962), but it did inhibit neurones in the cerebral cortex of this animal (Krnjević and Phillis, 1963). When injected into the septal region, it induced a catatonic-like state (Heath, Leach and Verster, 1962).

MH, injected into the cerebral ventricles, lacks the pharmacological effects of histamine (White, 1961b), but this does not rule out the possibility that catabolites of histamine may have an action on certain receptors in the brain (Green, 1964). McGeer, McGeer and McLennan (1961) found that ImAA was as active as gamma-aminobutyric acid in

inhibiting the crayfish receptor neurones.

The physiological significance of these findings remains to be explained. It is not even known to what tissues, vascular, neural or others, the occurrence and function of brain histamine is primarily related. A closer analysis of the effects of histamine on brain function is necessary. Study of the possible means of increasing or decreasing histamine concentration and its formation and catabolism in brain may shed more light on the functional significance of the amine. "It is tempting to assign functions to histamine extractable from particular regions of the brain, but until more is known about the exact site of its formation and storage and the conditions necessary for its release, speculation is likely to be unprofitable" (Adam,1961). "Nevertheless, histamine should be included among biologically active amines in the brain, such as catecholamines and 5-HT" (White, 1961) and "warrants consideration in any discussion of chemical transmission" (Crossland, 1960).

1/12 METHODS OF ESTIMATING HISTAMINE IN BRAIN

a) Chemical methods

The lack of useful absorption or emission properties of histamine (Lewis, Gebauer-Fuelnegg and Farmer, 1933) led to the development of methods which were based on the properties of derivatives.

1. Colorimetric method. This is based on the diazo reaction which was first introduced by Keossler and Hanke (1919) and later modified by Rosenthal and Tabor (1948). This method is neither specific for histamine nor very sensitive (lower limit of sensitivity: 500 ng in 5 ml solution). The values reported for rat brain histamine by this method were extremely high (Clouet, Gaitonde and Richter, 1956) (Table I).

2. Spectrophotometric method. This is based on the reaction with dinitrofluorobenzene (DNFB). It affords a simple and reliable procedure for the estimation of histamine (McIntire, White and Sproull, 1950). White (1966) used this method for the estimation of histamine in cat brain; he found that the lower limit of sensitivity was about 200 ng/g.

3. Spectrofluorometric method. This is based on the reaction with O-phthalaldehyde (OPT), first described by Shore, Burkhalter and Cohn (1959) and used for the estimation of histamine in brain. These authors reported that in 2 experiments out of 3, the results for the brain histamine obtained by bioassay and fluorometric assay agreed closely. They also observed that other imidazoles form fluorophors when condensed with OPT.

Carlini and Green (1963) discovered that butanol extracts of brain (Shore et al, 1959) contain a substance(s) that produces slow contraction of guinea pig ileum which is not prevented by mepyramine. This substance was removed when the extract was treated according to the method of Adam (1961) and may have been responsible for the apparent agreement between the results obtained by the two methods of estimation (Shore et al, 1959). Carlini and Green (1963) showed that values of histamine in rat brain by fluorometric assay were 4 times higher than those obtained with Adam's method (1961).

The fluorometric method is not specific for histamine since other substances are known to react

with OPT, e.g. various amino acids (Kremzner and Wilson, 1961) and diamines, particularly spermidine (Kremzner, 1965b; Kremzner and Pfeiffer, 1966; Michaelson, 1967). The method has undergone a number of modifications. Kremzner and Wilson (1961) used a strong anionic resin (Dowex-1 x 8) to remove histidine and other interfering substances from the extract. Biological assay of such brain extracts still gave values that were lower than those obtained by fluorometric assay (Kremzner, 1965a). However, when extracts are purified to meet the requirements of chemical assay, it does not follow that the purification is also suitable for biological assay; substances that do not react with OPT may nevertheless contract the guinea pig ileum and the reverse is probably true. Green and Erickson (1964) employed a strong cationic resin (Dowex 50) and found that histamine could be separated by gradient elution with HCl from interfering substances. McGeer (1964) applied brain extracts to a column of anionic resin (Dowex-I) and passed the effluent through a second column of cationic resin (IRC 50). Histamine was eluted with N-NaOH. Even with this purification procedure, occasional samples were obtained in which there was

severe quenching of the fluorescence of the internal standard histamine and/or an unusually high blank.

The presence of fluorophores other than the fluorophore derived from histamine was demonstrated by paper chromatography (Carlini and Green, 1963) and by isotope dilution methods (Levine, Sato and Sjoerdsma, 1965). Brain extracts to which ^{14}C-histamine was added were subjected sequentially to 3 procedures designed to separate histamine from interfering substances: (a) n-butanol extraction, (b) adsorption on IRC 50 resin and elution with acid and (c) passage through Dowex-I column. Aliquots of each extract were assayed by the method of Shore et al (1959) and radioactivity determined by scintillation spectrometer (Levine et al, 1965). It was found that specific activity of apparent histamine from brain increased with each step, indicating that each procedure removed some substance(s) other than histamine which reacts with OPT.

Despite various improvements in the method, it has still to be demonstrated that the final fluorophore obtained from extracts of brain derives only from histamine. Until this degree of purification can be

achieved, the fluorometric assay of histamine in brain remains doubtful.

b) Biological methods

A reliable and sensitive biological method for estimating small quantities of histamine was developed by Barsoum and Gaddum (1935). But even later modifications (Code, 1937a; Code and McIntire, 1956) were not sufficiently sensitive for the estimation of low values in the brain. When assaying extracts containing small amounts of histamine, the problem of obtaining suitable concentrations of the amine in contact with the gut has led to the development of special techniques, since neither great volumes of the extract can be added, nor can the bath be made conveniently smaller than 2 mls (Mongar and Schild, 1950). In order to solve this problem, advantage has been taken of the method devised by Finkleman (1930) in which a piece of intestine is suspended in air and kept alive by a stream of nutrient fluid running over its surface. Such a preparation was used for the assay of histamine by Kwiatkowski (1941) who injected the drug into the stream of fluid. Gaddum (1953) applied the term superfusion to this procedure and

obtained greater sensitivity by interrupting the flow of Tyrode's solution and applying the test solution, undiluted, directly to the surface of the muscle. Superfusion, however, is suitable only when the test solution is sufficiently pure and has a composition similar to the physiological fluid bathing the gut between the doses (Adam, 1961). Adam, Hardwick and Spencer (1954) described a semiautomatic device for the bioassay of histamine by superfusion. They were able to estimate quantities as small as 1 to 2.5 ng/ml. The error of the assay was no greater than that of the conventional method (index of precision, λ, mean 0.03, range 0.012 - 0.052, (12))

c) Enzymatic-Isotopic Method

Snyder, Baldessarini and Axelrod (1966) have suggested an enzymatic-isotopic method for the estimation of histamine in brain. Tissues are incubated with tracer amounts of ^{3}H-histamine and ^{14}C-S-adenosylmethionine in the presence of INMT, which specifically methylates histamine. The N-methylhistamine formed was extracted and the ratio ^{14}C to ^{3}H determined. The ratio of ^{14}C to ^{3}H was found to be directly proportional to the amount of unlabelled

histamine present in the incubation mixture. The relationship was linear over the range of 2 to 1000 ng. In every tissue examined, there was a single peak of radioactivity which corresponded in R_f to authentic 1,4-methylhistamine. The value for whole brain histamine in the rat was estimated to be 60 ± 10 ng/g, which agrees with the values obtained in this laboratory (Table 1). But the concentrations for the rabbit and guinea pig hypothalamus reported by the use of this method (100 - 200 ng/g) are much lower than estimates obtained by the biological procedure (Table 1).

SECTION 2

EXPERIMENTAL

Choice of animals. Adult male albino rabbits (New Zealand White) were used in these experiments. The body weights ranged from 1.5 to 4.3 Kg (mean 2.7 (194))

2/1 COLLECTION OF BLOOD SAMPLES

The ear was moistened with warm water and the hair gently shaved off with the aid of sterile disposable surgical blade (Swann-Morton B.S. 2982, size 22). The ear was dried with cotton-wool and warmed by heating with an electric light bulb. The skin in the vicinity of the lateral vein was coated with a thin layer of soft paraffin (MacArthy's Ltd. S5797). Heparin (Evans Medical 25000 i.u./ml, diluted 1/5 with saline), 500 i.u./Kg, was administered i.v. through a polythene cannula inserted into the marginal vein of the other ear. The vein was then nicked with the sharp blade and a sample of 0.05 ml of blood was collected from the surface of the ear with a siliconed blood pipette (capacity 0.1 ml, Gold-Emil-Line).

One ear was used for the collection of blood samples, the other for the injection or infusion of drugs through a polythene cannula. After the collection of blood, bleeding was arrested by applying a piece of cotton-wool to the bleeding point and clamping it with a hair clip.

2/2 HAEMATOCRIT

Blood samples were collected from the ear vein, as described in Section 2/1. Blood was allowed to flow freely into a 5-ml beaker from which it was aspirated with a polythene connection, attached to 2.5-ml disposable syringe (B-D Yale). The blood was introduced into a haematocrit tube (Gold-Emil-Line BS2554). The tube was centrifuged for 30 min at 2700 r.p.m. (at 4°C) and the volume of packed cells read.

The blood samples for the haematocrit were taken simultaneously with samples for the estimation of histamine. The aim was to record changes in the haematocrit which might have occurred after the i.v. infusion of large volumes of fluid. The blood histamine values obtained towards the end of infusion, were corrected for change in the haematocrit reading.

Control haematocrit values varied from 33 to 49 per cent (mean 41.3 (111)), which are very close to those reported for the rabbit by Albritton (1952) (mean: 41.5, range: 33 - 50%, quoted in 'Biology Data Book', p.269, 1964). After the infusion of various amino acids or saline the mean haematocrit value was 40% (range: 38 - 44% (84)).

2/3 PREPARATION OF AMINOACID SOLUTIONS FOR I.V. INFUSIONS

Amino acids were dissolved in sterile pyrogen-free distilled water. For simultaneous control experiments, sterile isotonic saline (0.9 per cent; 300 mosmoles/l) was used.

2/3/1 Histidine solutions

Neutralization with N/1 NaOH. On theoretical calculations 1 gram of histidine-HCl (m.w.191.62) would contain 5.2 mEq of HCl and hence would require the same amount of NaOH to neutralize it.

400 mg of histidine-HCl was dissolved in 10 ml distilled water. This solution was acidic (pH 4.0). On titration with N/1 NaOH (electrometrically), the volume required to bring the pH to neutrality was 1.7 ml. Hence it was calculated that 4.25 ml

(4.25 mEq) of N/I NaOH would be needed to neutralize I gram of histidine+HCl.

Neutralization with $NaHCO_3$. Histidine solutions for i.v. infusions were neutralized with solid $NaHCO_3$. On the basis of the above findings it was calculated that 360 mg of this salt (4.25 mEq) would be required to neutralize I gram of histidine-HCl.

Osmolarity of solution. Two limiting factors were considered in making up the histidine solution for i.v. infusion: (a) solubility of histidine in water (125 mg histidine-HCl/ml; 43 mg histidine base/ml at 25°C: from 'Biological Data', 1956), and (b) volume and osmolarity.

On the basis of the above calculations 40 mg of histidine-HCl would require for neutralization 14.4 mg of $NaHCO_3$ (0.17 mEq). It was calculated that the amount of NaCl formed in the reaction of 40 mg histidine-HCl and 14.4 mg of $NaHCO_3$ would be 0.17 mEq (9.9 mg of NaCl/ml water). Although this solution (approx. 30 mg base/ml) was found to be neutral (pH 6.8 to 7.2) and calculated to be nearly isotonic in respect to its NaCl content (9.9 mg/ml, equivalent to 340 mosmoles/litre), it was hypertonic (by 192 mosmoles*) when histidine molecules were taken into account. Hence the total osmolarity

---

* Concentration of histidine: 30 mg base/ml or 30000 mg/litre divided by 155 (m.w. of histidine base) = 192 mmoles/litre (equivalent to 192 mosmoles/l)

of the solution was 532 mosmoles per litre of solution (340 contributed by NaCl and 192 by histidine base). But in order to avoid infusing very large volumes of fluid, this solution (approx. 30 mg histidine base/ml) was not diluted any further.

A concentration of 40 mg histidine-HCl. IH_2O/ ml water (equivalent to 30 mg base/ml) was used for i.v. infusion of 500 mg/Kg/2 hr (16.7 ml/Kg) (See Table 7). When lower doses of histidine (5 to 250 mg/Kg) were infused, the volume was kept constant (16.7 ml/Kg/2 hr, Table 7). All these solutions were neutralized with the required amount of $NaHCO_3$ and made isotonic by the addition of NaCl as shown in the Table. The osmolarity contributed by histidine base and the NaCl formed was calculated. The number of mosmoles of NaCl needed to make the solution isotonic (300 mosmoles/litre) were converted to mg of NaCl at freezing-point depression of 0.56°C, by consulting the 'Documenta Geigy Scientific Tables', p. 327 (6th Edition).

2/3/2 <u>Other amino acids</u>

The solutions were freshly prepared on the day of experiment. The pH of the solutions, their neutralization, concentration and dosage, and the amount of NaCl required to make the solutions

Table 7

Summary of data used in the preparation of amino acid solutions for intravenous infusion

Amino acid*	mol. wt.	Solubility in water mg/ml	Dose for i.v. infusion, mg base/Kg/2hr.	Volume for i.v. infusion, ml/Kg/2hr.	Concentration, mg base/ml	pH	Volume of N/10 NaOH required to neutralize, ml/g amino acid	Dose in mmol/Kg	mosmoles/l contributed by amino acid	NaCl required to make solution isotonic (300 mosmoles/litre), mg/100 ml
L-HISTIDINE	155.12	43	500	16.7	30	4.0	(360 mg $NaHCO_3$ for every gram of histidine HCl)	3.20	192	(hypertonic)
			250	16.7	15			1.6	96	160
L-Histidine-HCl	191.62	125								
			125	16.7	7.5			0.8	48	570
L-Histidine-HCl. 1 H_2O	209.63									
			62	16.7	3.75			0.4	24	760
			30	16.7	1.875			0.2	12	850
			15	16.7	0.94			0.1	6	900
L-α-METHYL DOPA. $1\frac{1}{2}$ H_2O	211 (base)	10	200	16.7	12	4.2	0.5	0.95	57	750
L-DOPA	197	5	100	20	5	5.8	0.6	0.51	25	850
L-TRYPTOPHAN	204	11.4	200	16.7	12	5.8	0.2	0.98	59	760
DL-5-HYDROXY-TRYPTOPHAN	221		75	16.7	4.5	6.7		0.34	20	880

* Obtained from Koch-Light.

isotonic are summarized in Table 7. Osmolarity contributed by the amino acid and sodium ions (from NaOH) was calculated and NaCl was added in quantities sufficient to make the final solution isotonic with serum (300 mosmoles per litre).

When one of these amino acids was infused simultaneously with histidine, both were dissolved in the same vehicle. Both amino acids were neutralized (histidine with $NaHCO_3$; other amino acids with NaOH). Total osmolarity was calculated taking into account the amino acids present and NaCl formed.

2/4 INFUSION OF DRUGS

Amino acids used in the present work were infused intravenously at a constant rate over a period of about 2 hours, using a sterile disposable syringe (B-D Yale) with a polythene cannula and a slow injector.

The injector was previously calibrated with a 20-ml syringe to determine the rate of flow at various speeds. In each experiment the appropriate speed was employed to deliver the required dose of amino acid over a 2-hr period.

A polythene cannula of about I ft. in length was made for each infusion or injection. The polythene tubing (Sterivac, size 2) was drawn out at

both ends over a spirit lamp. The metallic part of a 23-gauge needle (B-D Yale) was removed from its plastic mount and fitted in one end of the tube. This end of the cannula was inserted into the marginal vein and secured in position by a narrow piece of adhesive tape; a needle (21-gauge) was fitted in the other end of the tubing and attached to the syringe.

2/5 PREPARATION OF THE RABBIT FOR THE REMOVAL OF BRAIN

The rabbits were sacrificed by bleeding under anaesthesia. Pentobarbitone sodium (Abbott) was injected slowly into the marginal vein through a polythene cannula. The dose of pentobarbitone varied from 34 to 78 mg/Kg (mean 53 mg/Kg (33)). Surgical anaesthesia was reached within 30 to 75 minutes.

The animal was then secured to a warm dissecting table with a block under its head. The hair overlying the neck region was shaved off with clippers and a mid-line incision was made in the neck. Both carotid arteries were exposed by blunt dissection and tied with ligatures. A bulldog clip was applied about I inch from the ligature and caudal to it. A small cut was made in the artery with fine spring scissors and a polythene cannula

introduced into the artery and secured in position by a ligature. Heparin (500 to 800 i.u./Kg) was injected i.v. through a cannula, and bulldog clips were released from the carotid arteries allowing the blood to flow freely into a measuring cylinder. The volume of blood collected varied from 50 to 130 ml (mean 90 ml (38)).

In some of the control and treated rabbits, the head was perfused with Ringer-Locke solution (S.2/6).

2/6 PERFUSION OF HEAD WITH RINGER-LOCKE SOLUTION

The aim was to perfuse the head through both carotid arteries so as to remove as much residual blood as possible from the cerebral vessels. The concentration of histamine in hypophysis and different parts of the brain, collected after head perfusion, were compared with the values obtained from rabbits which were only heparinized and bled, but not perfused. The fact that rabbit platelets are rich in histamine has already been referred to in Section (L/5). These experiments were intended to test the extent to which residual blood might contribute to histamine in brain and hypophysial extracts.

Immediately after bleeding, the rabbit's head was perfused with 300 ml Ringer-Locke solution.

The solution flowed from a I-litre aspirator which was connected by a Y-piece to two polythene tubes (Sterivac, size 2), which were introduced into the carotid arteries pointing rostrally (the carotid arteries having been already ligated caudal to point of insertion). Solution was then allowed to flow freely through the carotid arteries, thus perfusing the head. The effluent returned by way of the jugular veins, which were cut. The pressure head was about 52 inches and the flow rate 30 ml/min. At the end of perfusion the effluent was almost clear. When the brain was removed under these conditions, it was pale and soft.

2/7 REMOVAL OF BRAIN AND HYPOPHYSIS

The skull was exposed by a mid-line incision and an opening was made with a trephine. The upper part of the skull was removed with bone nibblers, leaving the dura mostly intact. The tentorium was carefully removed.

The dura was cut and reflected. The olfactory lobes were divided and the brain was then gently lifted; the remaining cranial nerves were cut and the hypophysial stalk was severed. The spinal cord was divided at C 2. The time taken from the death of the animal to the removal of brain was about 7 minutes. The brain was weighed and immediately

placed on a cold plate. The hypophysis was removed by cutting the dura behind and at the sides of the gland. The weight of the brain ranged from 8.2 to 12.9 g (mean 9.7 g (46)). Dissection was begun without delay.

2/8 DISSECTION OF THE BRAIN

The hypophysis was cleared from the surrounding connective tissues and the anterior lobe was separated from the posterior lobe. The pia-arachnoid on the ventral surface of the brain was stripped off. The brain was then divided transversely along a line passing between the pons and the inferior colliculi. The anterior part was further divided into right and left halves by a mid-sagittal section.

Samples were usually collected from the right half of the brain and are described as follows:

Anterior lobe. Contained mainly the pars distalis of the adenohypophysis.

Posterior lobe. Contained mainly the infundibular process and the pars intermedia of the adenohypophysis.

Hypothalamus. Included the corpora mammillaria, the preoptic region and the ventral and dorsal hypothalamus. A rectangular block of tissue was cut to a depth of about 3 mm, and usually medial to

fibres of the fornix which end in the corpora mammillaria (Fig. 1).

Medial thalamus. Taken from the region of massa intermedia. A rectangular block of tissue was cut to a depth of about 3 mm (Fig. 1).

Superior and inferior corpora quadrigemina. Removed by cutting along their bases (Fig. 1).

Region of the reticular formation. Was the region in the pons-medulla or the tegmentum of the midbrain (Fig. 1).

Central grey matter. Corresponded to the substantia grisa centralis, and is the mass of grey matter which surrounds the aqueduct. A block of tissue was cut on one side of the aqueduct to a depth about 2 mm (Fig. 1).

Cerebral cortex. A sample of the grey matter only was cut from the temporo-parietal region.

Cerebellum. Taken from the vermis.

Caudate nucleus. About 2/3rd of the region was removed.

Hippocampus. Taken as a transverse segment.

Floor of the 4th ventricle. Was the grey matter cut to a depth of about 2 mm.

Choroid plexus. Taken from the lateral ventricle.

In all 19 different areas were sampled, including those of the hypophysis. The samples were

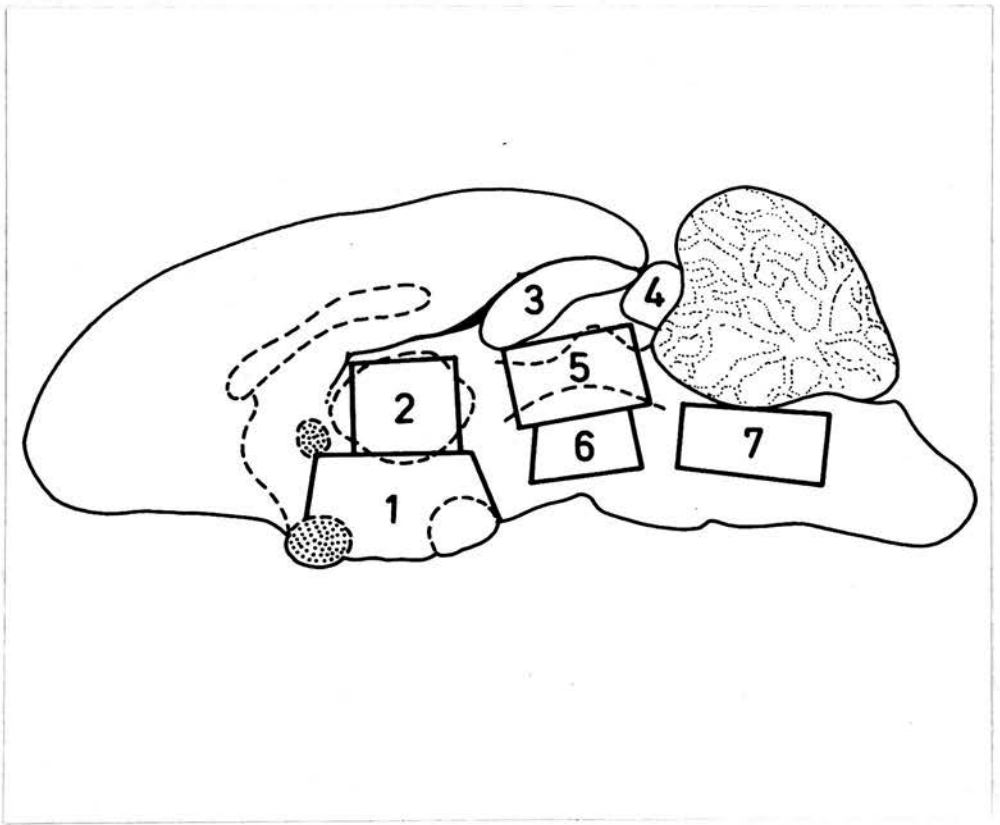


Fig. I. Medial sagittal section of rabbit's brain. Demarcation of samples: (1) hypothalamus; (2) medial thalamus; (3) superior corpus quadrigeminun; (4) inferior corpus quadrigeminun; (5) central grey matter; (6) region of tegmentum; (7) pons-medulla.

obtained from a total of 46 rabbits.

2/8/1 Weighing of samples

The sample was weighed without delay on a torsion balance of 100 mg capacity using a wire basket. The time required for the collection of samples was about 30 min. The weight of any particular sample varied from rabbit to rabbit (Table 13), partly because of the differences between rabbits but also because of the error of dissection. In order to reduce this error, the dissection of the brain was usually done by the same person.

2/9 EXTRACTION AND PURIFICATION OF HISTAMINE

Adam, Hardwick and Spencer (1957) developed a method of estimating histamine in plasma. Adam (1961) modified this method for the estimation of histamine in different parts of dog's brain. This method with minor modifications was used by Adam and Hye (1966). In the present work this method was employed for the estimation of histamine in the hypophysis, brain and blood.

2/9/1 Apparatus

Tissue grinders. These were made of 15 ml heat-resistant conical centrifuge tubes (MSE Cat. No. 69353). The lower part was ground and fitted

with a corresponding pestle, made of soft glass. The tube was calibrated to contain 5 ml.

For estimation of histamine in the whole brain of the rabbit and other gross areas, larger homogenizers were used (Fisons, TTM/20).

Glass tubes for column chromatography. Columns were made in glass tubes (Fig. 2) which were 13-14 cm in length and 8.0 mm in internal diameter. The tube was surmounted by a bulb of 25 ml capacity and closed by a glass tap. The overall length was 27-28 cm. The dead space below the column (tap and capillary below it) was about 0.3 ml. A plastic cap covered the top of the bulb. Nine such tubes were held up by spring clips on a dexion frame.

Refrigerator centrifuge. Type MSE "Medium".

pH Meter. (Beckman Zeromatic).

2/9/2 Chemicals and solutions

Chemicals used in the experiments were of analytical grade unless stated otherwise. Fresh, glass distilled water was used for solutions and elsewhere in the procedure.

Ion exchange resin. Amberlite, CG 50, Type 1 100-200 mesh (Rohm and Haas Co., Philadelphia).

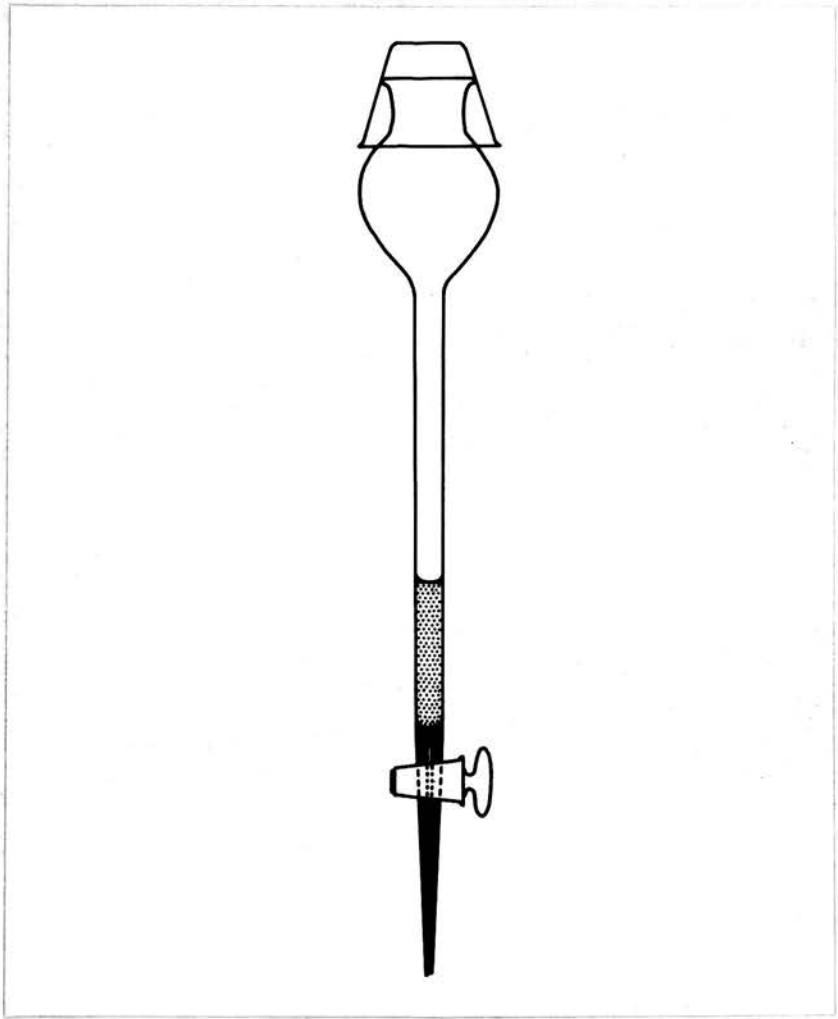


Fig. 2. Glass tube for column. For details see text.

The resin is a weak cation exchanger with carboxylic (-COOH) groups. It is supplied in the acidic (H) form.

Cellulose powder. Whatman Standard Grade (CF-II, Cat. No. IIIII) for chromatography.

Trichloracetic acid (BDH). 6 per cent w/v solution in water (TCA). The sclution was filtered.

Hydrochloric acid. 0.I N and 0.25 N HCl prepared from the concentrated volumetric solution (BDH). 6.0 N HCl prepared from concentrated HCl (36 per cent HCl, BDH).

Sodium hydroxide. 0.INNaOH prepared from sodium hydroxide pellets (BDH) and titrated against standard 0.I N HCl, using phenolphthalein as internal indicator.

Neutral red (BDH). 0.0I per cent (w/v) in water.

Phosphate buffer: 0.2 molar; Na^+ concentration 390 mEq/litre. 15.6 g NaH_2PO_4. 2 H_2O dissolved and made up to 500 ml with water = Solution A.
28.4 g Na_2HPO_4 dissolved and made up to I litre with water = Solution B.
26.5 ml of Solution A was mixed with 473.5 ml of Solution B to make 500 ml of buffer solution. The

pH of this solution was 7.92 and the total Na^+ concentration was calculated as 390 mEq/litre.

Phosphate buffer: 0.05 molar; Na^+ concentration 100 mEq/litre. 256.0 ml of 0.2 molar buffer (390 mEq/l Na^+) was made up to I litre with water. the pH of this solution was 8.0 and the total Na^+ concentration was calculated as 100 mEq/litre.

Chromic-sulphuric cleaning solution. 70.0 g/l of sodium dichromate in concentrated sulphuric acid (commercial grade).

2/9/3 Procedure

Cleaning of glassware. Glassware was left in cleaning solution for at least 2 hr. Later it was rinsed thoroughly, first in warm running water and then six times with distilled water. Apparatus in contact with concentrations of histamine above 100 ng/ml was soaked in 2N NaOH for at least 24 hr being before/finally cleaned with chromic-sulphuric solution.

Protection of glassware. Much of the clean, dried glassware was stored in boxes to protect it from dust in the air. Pipettes used for measuring volumes of reagents or solutions in the procedure were stored individually in stoppered glass tubes,

thereby reducing the risk of cross contamination.

Preparation of resin. 30 g of resin was suspended in I.0 litre of distilled water in a beaker and allowed to sediment for 10 min. The water was decanted. The procedure was repeated 6 times. The final deposit was transferred to a porcelain dish and dried at 45°C for 48 hr. The cake was broken up with a small pestle. The resin prepared in this way was free from very fine particles.

Preparation of columns. 50.0 mg of the prepared resin was mixed with 300 mg of cellulose powder in a 25 ml conical flask. 4.0 ml of 0.I N NaOH was added to the flask and the contents were mixed by gentle shaking. The resin-cellulose mixture was left overnight at 4°C for use on the next day. The resin was thereby partly converted into sodium form.

The tube was cleaned with chromic-sulphuric solution immediately before use. A pad of glass wool was placed at the bottom of the tube and the tap was lightly greased with yellow soft paraffin. The tube was rinsed from inside, first with distilled water and then with the buffer solution (0.05 molar, 100 mEq/l Na^+).

5 ml buffer solution (0.05 molar, 100 mEq/l Na^+) was placed in the flask containing the resin-cellulose

suspension. The contents of the flask were well mixed and poured into the tube. The tap was fully opened and the resin-cellulose mixture was allowed to form a column. When the liquid level reached the top of the column, the tap was closed. The height of the column was about 42 mm. The volume occupied by the column was about 2 cm^3.

Equilibration of column. 20 ml of buffer solution (0.05 molar, 100 mEq/l Na^+, pH 8.0) was applied to the column, and care was taken to wash down the inner side of the tube. The buffer was allowed to flow at a rate of about I ml in every 3 min, by adjusting the tap. The column was equilibrated in this way for I hr, at the end of which it was ready for use.

Nine columns were usually prepared. Eight of these were for the tissue extracts and one was used as a control.

Extraction of tissue sample. The sample was removed from the weighing basket with the aid of a fine glass rod and lowered into a measured amount of TCA in the tissue grinder. The volume of TCA used was 5 µl/mg of tissue. The tissue was immediately ground and the process continued with

the addition of a small volume (I ml) of water. The final volume in the tube was made up to 5 ml with water. The suspension was thoroughly mixed and the tube was sealed with parafilm. Usually 8 samples were extracted at a time.

When the whole brain or large areas of the brain were extracted, the tissue was placed in a large homogenizer (Fisons, TTh/20) containing the required volume of TCA. The tissue was ground using a motor-driven stirrer (Griffin and Tatlock). The pestle and the homogenizer were washed with distilled water and the suspension of the precipitate was poured into a glass-stoppered measuring cylinder. The volume was made up with distilled water so that 12 mg of tissue was contained in I ml of extractant. 5 ml aliquots were collected (equivalent to 60 mg of tissue), after a gentle mixing of the suspension.

Centrifugation of the extract. The tubes were centrifuged at 2000 r.p.m. at 4°C for 30 min.

Collection of aliquots. An aliquot of 4.6 ml was removed from clear supernatant using a Pasteur pipette. The aliquot was placed in a stoppered, graduated 10 ml tube. The precipitate was discarded.

Preparation of solution for adsorption.

Neutralization of the aliquot. The aliquot of the supernatant was neutralized with 0.I N NaOH delivered from 1 ml burette. One drop (0.04 ml) of neutral red solution was used as internal indicator. Approximately 0.02 ml of alkali was required for each mg of tissue. After the point of neutralization had been reached, one drop (0.02 ml) of 0.I N HCl was added from another burette to make the solution just acidic.

Adjustment of pH and Na^+ concentration in the solution. The neutralized aliquot was brought to pH 8.0 and to a total Na^+ concentration of approximately 100 mEq/l by the addition of phosphate buffer (0.2 molar, 390 mEq/l Na^+, pH 7.92). Water was then added to a final volume of 8.0 ml. The volume of buffer depended on the quantity of Na^+ added in the neutralization (Na^+ derived from the tissue was neglected).

In the hypothetical case where the aliquot does not require the addition of 0.I N NaOH (i.e. the solution is already neutral), the volume of buffer (0.2 molar, 390 mEq/l Na^+) for the adjustment is calculated as follows

$$\frac{8 \text{ ml} \times 100 \text{ mEq/l}}{390 \text{ mEq/l}} = 2.05 \text{ ml}$$

When alkali was added, the volume of buffer required was less than 2 ml. The exact volume of buffer was calculated as follows:

Na^+ present in 1.0 ml of buffer (390 mEq/l Na^+)

= (390 x 23) / 1000=8.97 mg

Na^+ present in volume (ml) of 0.1 N NaOH added

= (2.3 x ml of 0.1 N NaOH added) mg

Volume of buffer (390 mEq/l Na^+) to be added

$$= \frac{(8.97 \times 2.05) - (2.3 \times \text{ml of 0.1 N NaOH added})}{8.97}$$

A calibration curve was drawn on the basis of this calculation. For a given volume of 0.1 N NaOH, the required volume of buffer could be read off directly from the graph. After addition of the buffer the volume in the tube was made up to 8.0 ml with water. The solution was mixed and was ready for transfer to the column.

Adsorption and elution.

Adsorption. 8.0 ml of the buffered aliquot was applied to the equilibrated column. The graduated tube was washed with 2.0 ml of buffer solution (100 mEq/l Na^+, pH 8.0) and this was placed on the column. The control column received 10 ml of buffer solution (100 mEq/l Na^+, pH 8.0).

The flow was controlled at the rate of 1 ml in 3 min. The effluent was collected in a beaker.

Histamine has two pK_a values: one due to imino nitrogen in the imidazole ring is 5.9 and the other due to the amino group is 9.7-9.8 (Levy, 1935; Holmes and Jones, 1960). At pH 8.0 nearly all the histamine (98.04%) present in the solution would be ionized. Adsorption of histamine by the column at pH 8.0 is complete (Adam et al, 1957).

Wash. After the adsorption step, the column was washed with 5.0 ml water, which was allowed to flow at the rate of about I.0 ml in 3 min. The effluents were discarded.

Elution. The elution was carried out with 2.0 ml of 0.25 N HCl, followed by 3.0 ml water. The eluate was collected in a stoppered glass tube (Quickfit, MF 24/3, B 24/29, capacity 50 ml). The flow rate during the elution was reduced to about I ml in every 4 min. The neutral red remained adsorbed at the top of the column.

Hye (1964) analysed the eluate fractions for the pH values and for the amount of Na^+ and histamine present.

Evaporation of the eluate. The eluate was dried at 55°C under reduced pressure (20 to 30 mm Hg). The evaporation was done in a thermostatically controlled water bath, the tube containing the

solution being connected to a high pressure water pump through an adaptor. The vacuum was gradually reduced when the residue was seen to be still slightly moist, to prevent loss from flaking. The residue contained mainly NaCl derived from the column.

Heating with strong HCl. I ml of 6 N HCl was added to the tube and the tube was heated for 30 min. in an oil bath at 100°C (Adam, 1961). The tube was immersed in the oil only up to the liquid inside. The stopper was loosened and left in situ. Under these conditions the HCl refluxed in the tube without detectable loss of vapour.

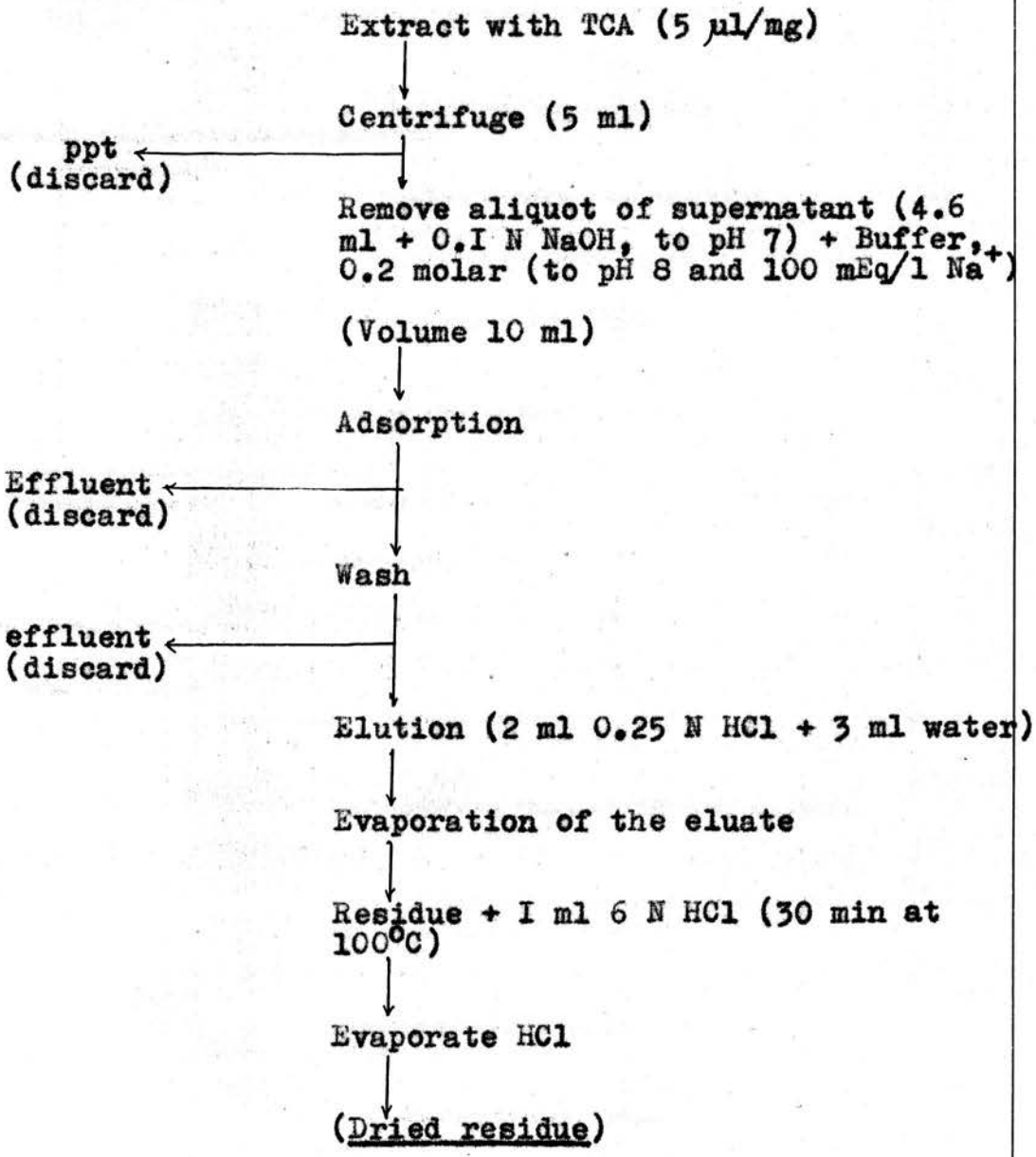
The acid was evaporated at 60°C under reduced pressure (20-30 mm Hg). Flaking was not observed at this stage and complete drying was possible. The neck and the stopper of the tube were dried with a stream of hot air to remove traces of HCl. (At this stage the tubes were generally stored overnight or longer).

Final drying of the residue. The last traces of acid were removed from the residue by heating the tube in a water bath at 80°C for 10 min and under reduced pressure (20-30 mm Hg). The neck of the tube and the stopper were again dried with hot air.

The contents of the tube, which will be referred to as the dried residue, were then ready for bioassay.

The dried residue contained about 24 mg of NaCl (Adam, 1961; Hye, 1964). Total potassium in dried residue obtained from brain tissue extracts by Hye (1964), ranged from 110 to 150 µg (from samples weighing from 50 to 70 mg). Presence of excess quantities of K^+ in the Tyrode's solution, could interfere with the assay of histamine by superfusion (Adam et al, 1954). The K^+ present in the tissue would probably be adsorbed on the column and eluted with HCl. Hye's results (1964) showed that the amount of K^+ present in samples weighing about 50 mg would hardly interfere with the assay of histamine.

A flow sheet of the procedure for extraction and purification of histamine for bioassay



2/10 BIOLOGICAL ASSAY

The assay was carried out by superfusion on the guinea pig ileum (Gaddum, 1953), using a semiautomatic apparatus as described by Adam et al, (1954).

2/10/1 Solutions

Chemicals of analytical grade were used.

Atropinized Tyrode's solution. The composition of the solution is given below. This solution is referred to in the text as Tyrode's solution.

	Stock solution g/l	Tyrode's solution g/l
NaCl	80.0	8.0
KCl	8.0	0.20
$CaCl_2$	5.5	0.14
$MgCl_2$	4.0	0.10
$NaH_2PO_4.2H_2O$	2.0	0.05
$NaHCO_3$	36.0	0.36
Glucose		1.0
Atropine sulphate (10^{-4})		10-7

The concentration of $CaCl_2$ and $MgCl_2$ in the stock solution was adjusted by titration against 0.I N $AgNO_3$, using 0.04 ml of potassium chromate (5 per cent w/v) as internalindicator.

Histamine standard solutions. The material used was histamine acid phosphate (BDH, m.w. 307.15). Values for histamine in the text refer to the base (m.w. III.I5), unless stated otherwise.

Stock histamine standard, 5 µg/ml in water. The solution was kept frozen at -17°C and used from time to time for periods of 2 to 3 months. Solutions kept in this way showed no loss of activity when compared with solutions prepared from salt on the day of the assay. The following solutions were prepared in Tyrode's solution at the time of assay.

a) 100 ng/ml. 2.0 ml of 5 µg/ml solution made up to 100 ml with Tyrode's solution.

b) 10 ng/ml. 20.0 ml of the 100 ng/ml solution made up to 200 ml with Tyrode's solution.

c) 3 ng/ml. 6.0 ml of the 100 ng/ml solution made up to 200 ml with Tyrode's solution.

d) Ten assay standards, 0.25 to 2.5 ng/ml. Prepared from the 10 ng/ml solution in the following way, (See following page).

From 10 ng/ml solution with 10 ml graduated pipette	Made up with Tyrode's solution in volumetric flasks	Concentration of Standard solution
1.25 ml	to 50.0 ml	0.25 ng/ml
2.50 ml	"	0.50 "
3.75 ml	"	0.75 "
5.00 ml	"	1.00 "
6.25 ml	"	1.25 "
7.50 ml	"	1.50 "
8.75 ml	"	1.75 "
10.00 ml	"	2.00 "
11.25 ml	"	2.25 "
12.50 ml	"	2.50 "

Modified Tyrode's solution. This was for reconstitution of the dried residue. The concentration of NaCl in this solution was only 3.65 mg/ml.* The solution was made up from a 'Stock Solution A' in the following way. (See following page).

* This was to compensate for the presence of about 24 mg of NaCl in the dried residue. When the dried residue was dissolved in 5 ml of this solution, the composition of the final solution (test solution) became close to that of Tyrode's solution. With the concentration used, the test solution could be slightly hypertonic compared with the Tyrode's solution. If the extreme values of NaCl present in the dried residue were 22 and 25 mg (Hye, 1964), on reconstitution the test solution would not differ by more than + 5.0 or -3.0 per cent, as compared with the Tyrode solution for the total NaCl. Such differences would not affect the bioassay by superfusion (Adam et al, 1954).

		Tyrode stock	
To make 200 ml 'Stock Solution A' from Tyrode stocks	KCl	(8.0 g/l)	50.0 ml
	$CaCl_2$	(5.5 g/l)	50.0 ml
	$MgCl_2$	(4.0 g/l)	50.0 ml
	$NaH_2PO_4.2H_2O$	(2.0 g/l)	50.0 ml

5.0 ml of the 'Stock Solution A' was diluted with water and 0.5 ml of $NaHCO_3$ solution (Tyrode stock, 36.0 g/l). The volume was made up to 50.0 ml with water. Finally the volume was made up to 92.0 ml by adding 42.0 ml Tyrode's solution.

The dried residue in the tube was dissolved in 5.0 ml of this solution. Any further dilution of the test solution was carried out with the Tyrode's solution only.

Mepyramine-Tyrode's solution. A stock solution of mepyramine maleate, 10 µg/ml (Base) in water, was kept frozen at -17°C. When required 1.0 ml of this solution was made up to 100 ml in Tyrode's solution to make 100 ng/ml solution of mepyramine.

15.0 ml of the 100 ng/ml mepyramine solution was made up to 500 ml with Tyrode's solution to make Mepyramine-Tyrode's solution containing 3 ng/ml (3×10^{-9}) of mepyramine.

Superfusion Apparatus

The apparatus used was similar to that described by Adam et al (1954) as shown in the diagram (Fig. 3). The various parts were mounted vertically on a strip of Dexion. In the present apparatus the 'doser' (F) was operated by a solenoid relay (R.A. Webber Ltd., Bristol, Type SCM for 24 volts D.C.) which was placed immediately above the plunger, to which the solenoid core was fixed by a coupling device. The plunger was made of steel wire, enclosed in a polythene tube (2 mm o.d.) which was drawn out to a closed fine point. This served as a valve at the opening of the 'doser' which was a 1-ml glass tuberculin syringe. The 'doser' was calibrated to contain 0.6 ml. The weight of the solenoid core was sufficient to keep the valve closed when the solenoid was inactive. The height to which the plunger would be lifted on activation of the solenoid was adjustable. This adjustment made it possible to widen or narrow the valve and thereby to control the flow rate of the dose. The connections to the solenoid were through a variable potentiometer (100 Ohms) which served to control the energy supplied to the relay.

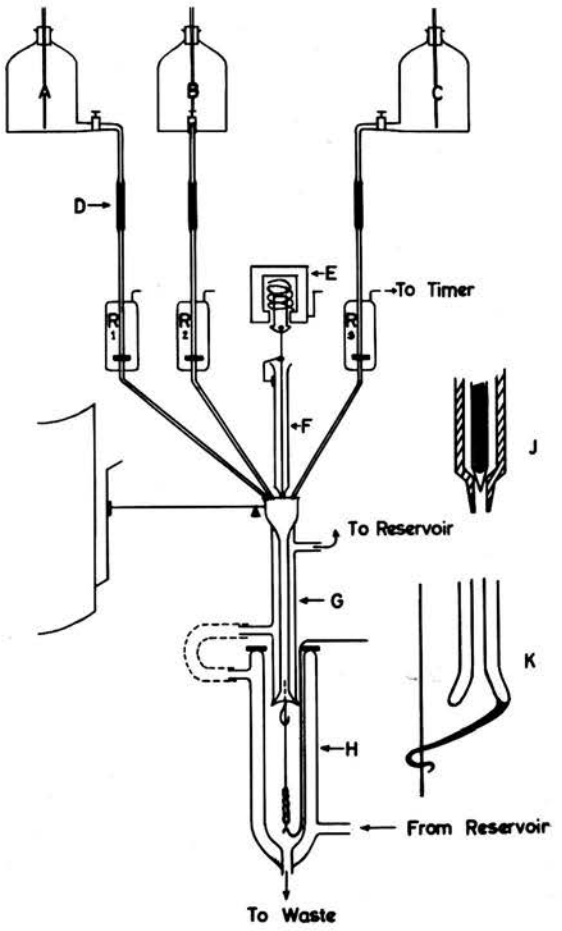


Fig. 3. Superfusion apparatus: (A) Tyrode's solution; (B) Tyrode + histamine; (C) Tyrode + mepyramine; (D) capillary resistance; (E) solenoid relay; (F) 'doser' with valve; (G) warming tube; (H) superfusion chamber; (J) lower end of 'doser' with valve; (K) lower end of warming tube with glass hook and thread in position. The timing unit and thermostatically controlled water reservoir are not shown in the figure.

The dose (0.6 ml) flowed over the gut in 8 to 9 seconds. The flow of Tyrode's solution returned I to 2 seconds later. The gap between these two flows was kept as short as possible to avoid spontaneous contractions of the gut. The point of maximal contraction after a dose of histamine was reached before the flow of Tyrode's solution had returned.

The pre-heating tube (G) was flared at its inlet and at the outlet to prevent retention of fluid by capillary action. The rim at the outlet carried a short glass arm which ended in a hook (K). Warm solution flowed along this arm: drops formed on the hook, fell along the enclosed thread and broke on the gut to enclose it in a film of liquid. The drops re-formed at the end of the gut and went to waste.

Relays I, 2 and 3 were P.O. type relays (100 Ohms). A Mariotte bottle (A) of 2 litre capacity served as the reservoir for Tyrode's solution. The rate of flow from the bottle was controlled by a capillary resistance (D) which allowed a flow of 4.6 ml/min. This amounted to 60 drops per min. on the gut. The flow stopped with the action of Relay I.

A Mariotte bottle (B) of 500 ml capacity contained the histamine solution (3 ng/ml), which flowed

through a capillary resistance at the same rate as the Tyrode's solution, but only when Relay 2 was active. This dose was delivered automatically and served to sensitize the gut to histamine before starting the assay. When either the solenoid relay (E) of the 'doser' or Relay 2 was active, the flow of Tyrode's solution was automatically stopped by the simultaneous action of Relay I. The solenoid relay and Relay 2 were connected by a two-way switch so that when one of the two was active, the other was disconnected from the circuit.

A Mariotte bottle (C) of 500 ml capacity contained Tyrode's solution with mepyramine (3 ng/ml) when this was used. The flow of this solution through a capillary resistance was at the same rate as that of Tyrode's solution (i.e. 4.6 ml/min). Relay 3 served the same purpose as Relay I and so stopped the flow of mepyramine-Tyrode's solution while a dose was flowing over the gut.

The control of the dose cycle was through a Sequential Timing Unit as described by Austin (see Adam et al, 1954).

The conditions of the assay can be summarized as follows:

Dose cycle ..... 72 sec

Time for the dose to flow over the gut ... 8 sec

Solenoid relay of doser activation for ... 10 sec

Flow rate

Tyrode's solution ..... 4.6 ml/min (= 60 drops on gut)

Dose (0.6 ml) ..... 8-9 sec

Temperature

Water bath reservoir ..... 34°C

Inside superfusion chamber ..... 33°C

Flow at the outlet of warming tube ..... 32°-33°C

Lever: (light balsa wood)

Total length ..... 27.5 cm

Magnification ..... 8.4 times

Fixed tension ..... 500 mg

Variable load ..... 210 mg

Writing point: frontal, made of capillary glass tube

2/10/3 Guinea-pig ileum

A strip of the terminal part of the ileum, 3 to 4 cm long when slightly extended, was taken from young guinea-pigs weighing 150 to 300 g. As compared with that of heavier animals, the gut was usually thinner and showed less spontaneous activity but became more readily sensitive to small doses of his tamine.

2/10/4 Procedure of assay

Sensitization. The gut was left under superfusion with only Tyrode's solution flowing over it for at least 30 min. The histamine standard solutions were prepared and sensitization of the gut with 3 ng/ml histamine solution was started. The timing Unit was switched on with connections to Relay I and 2. The dose cycle started with doses of 3 ng/ml histamine once every 72 sec. The drum was kept at a low speed (2 mm/min). The paper was very lightly smoked. With the first few doses the gut usually showed increasing sensitivity but soon the contractions became of similar magnitude and an equilibrium was reached. The base line normally remained stable and straight but varied to some extent with very sensitive guts. When the gut was found to be poorly sensitive, it usually improved with the passage of time and it was seldom necessary to change it for another strip.

Dose-response curve. When the gut was sufficiently sensitive and stable, a dose-response curve was recorded with histamine standard solutions from 0.25 to 2.5 ng/ml. The doses were placed in the 'doser' by hand using separate

Pasteur pipettes*. In the range 0.25 to 2.5 ng/ml of histamine, the dose-response curve was approximately linear and the gut usually detected differences of 0.25 ng/ml in this range (Fig. 4). Often both an ascending and descending dose-response curve was recorded. A lower dose followed by a much higher one (or the reverse) sometimes affected the response, owing to residual solution in the 'doser'. Interference from this cause within a sequence of doses seldom occurred because it was always possible to perform the assay within a narrow range of the dose-response curve. When Tyrode's solution was applied from the 'doser' (in place of a dose), there was usually a small contraction of the gut. This was always less than the concentration with 0.25 ng/ml histamine standard and was regarded as a 'zero effect'.

Test solution. The dried residue was dissolved in 5.0 ml of the modified Tyrode's solution (S.2/10/1). Since many of the test solutions required further dilution, the first dose was a trial fractional dose of the test solution. 0.2 to 0.3 ml of

* These were special long Pasteur pipettes (about 30 cm) which were drawn out and bent at the tips. About 2 ml of solution could be withdrawn conveniently from a 50 ml flask. Similar pipettes were used for applying the test solution on the 'doser'.

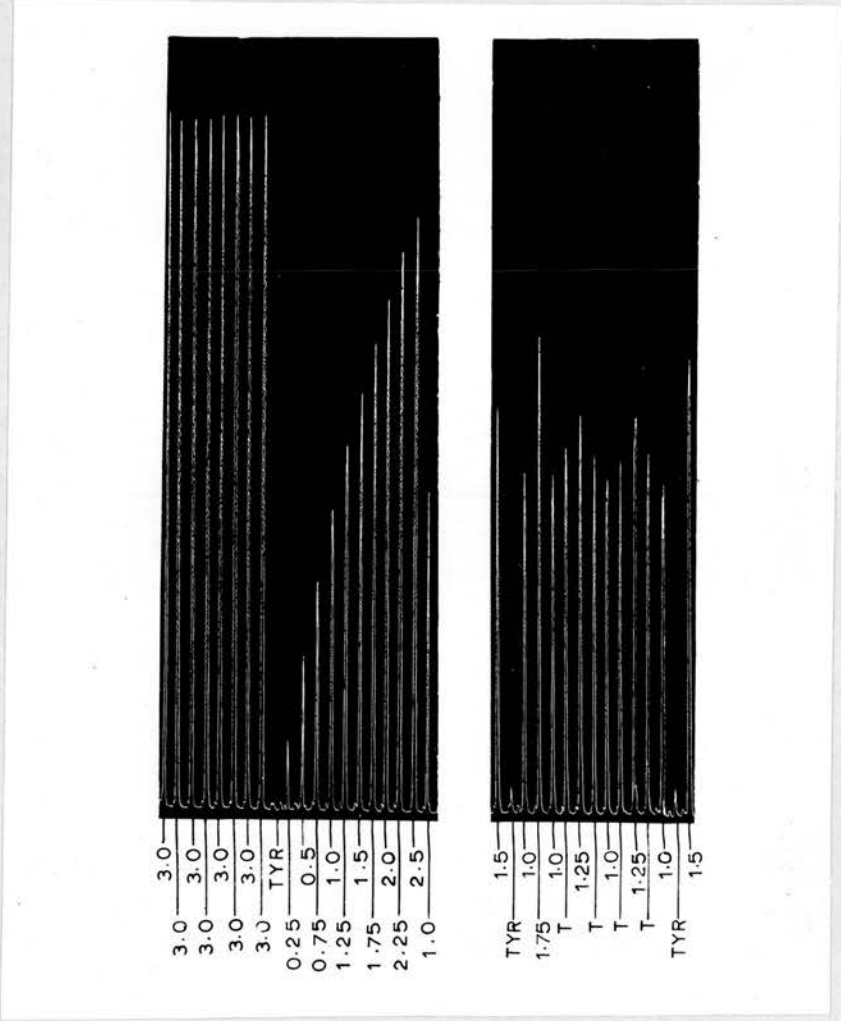


Fig. 4. Responses of superfused guinea pig's ileum to doses of histamine standard solutions, Tyrode's solution and a purified extract of brain. The first part of the tracing shows a dose-response curve and the second part a typical assay. The numerals give doses of histamine standard solutions in ng/ml. Other doses are labelled as follows: TYR, Tyrode's solution; TF, a fractional dose of the test solution; T, test solution diluted 1 in 4 and estimated to contain 1.12 ng/ml.

the test solution was placed in the 'doser' and Tyrode's solution from a wash bottle was added in the 'doser' up to the mark at 0.6 ml. The proper dilution of the test solution, required for the assay could be inferred from the contraction with the trial dose. When required, a dilution of the test solution was made in a 10 ml graduated cylinder.

The assay. This was done by bracketing. An arithmetical scale for the standard doses was chosen (Adam et al, 1957). Usually 5 or 6 doses of the test were interposed between doses of the standards. A dose of Tyrode's solution was given between two sets of assays.

The estimate of concentration of histamine in a tissue sample w as calculated as follows:

Estimate of concentration (ng/ml) in the test solution X dilution factor X 5 X 5/4.6

= Estimate as nanogram (ng) in whole tissue sample.

$$\frac{\text{ng of histamine in sample} \times 1000}{\text{weight of sample in mg}}$$

= Estimated concentration of histamine ng/g of the tissue sampled.

The factor 5/4.6 was used to correct for the volume of aliquot (4.6 ml) collected from the supernatant, assuming that the histamine present in the

extract was equally distributed in the supernatant and in the much smaller volume occupied by the precipitate.

2/10/5 Mepyramine test.*

In testing for the presence of histamine in commercial samples of histidine, the test solution was reassayed in the presence of Tyrode's solution containing 3 ng/ml mepyramine. A few doses of 3 ng/ml histamine were applied at first. The contractions to 3 ng/ml histamine soon became minimal. Doses of the test were then applied alternating with equiactive doses of histamine standards (those used beforehand for bracketing the test). The test was reassayed.

2/11 RECOVERY EXPERIMENTS

The object of these experiments was to test the efficiency of the method.

a) Histamine added to brain (in quantities of 25, 50 and 100 ng)

I. Histamine was added to fresh samples of cerebellum (50-60 mg) after precipitation with TCA (5 µl/mg).

2. Histamine was added to 4 ml aliquots of a suspension of precipitate obtained by adding

* A more complete Mepyramine test was reported by Adam (1961) and Hye (1964). It was found that a concentration of mepyramine which abolished the response of the ileum to equiactive doses of test and histamine s tandard, only slightly reduced the response to doses of 5-HT and substance P (Adam, 1961).

TCA (5 µl/mg) to cerebral cortex; the precipitate was then suspended in water so that the final amount of tissue in the extractant was 15 mg/ml. Each aliquot contained 60 mg of tissue.

In all these experiments control samples were taken to estimate the histamine originally present in the tissue. The recovery was calculated from the difference between the total amount estimated and that present in the control.

b) Histamine added to supernatant of brain extracts

The same quantities of histamine were added to the supernatant obtained from cerebellar tissue (see page 55) after precipitation with TCA, centrifugation and neutralization. The recovery was calculated as in the previous experiments.

c) Histamine added to buffer solution for adsorption

To test for the loss of histamine after adsorption step (see page 57), the same amounts of histamine were added to 10 ml aliquots of buffer solution (0.05 molar, 100 mEq/1 Na^{+}). The recovery of histamine from buffer was thus compared with the recovery from tissues as well as from the supernatant.

In two experiments 500 and 1000 ng of histamine

were applied to the columns with buffer solution to test for any loss of histamine in the effluent.

2/12 SPECIFICITY OF THE METHOD

The method has already been thoroughly tested in this laboratory (Adam, 1961; Hye, 1964).

The eluate retained its activity after heating in 6 N HCl for 30 min (Barsoum and Gaddum, 1935). This treatment destroyed the activity of 5-HT, noradrenaline, substance P and posterior pituitary extract (Adam, 1961).

On repeating the assay in the presence of low concentration of mepyramine (3 ng/ml Tyrode's solution) the estimates for histamine equivalent agreed to within 10% (Adam, 1961; Hye, 1964). These workers found that doses of test and standard solutions of histamine were always found to be equally inhibited or abolished by mepyramine.

An eluate from dog's hypothalamus lost 95% of its activity when incubated with histaminase (Adam, 1961).

Although Adam's method (1961) cannot discriminate between histamine and its catabolic methylated derivatives (Schild, 1947; Werle and Palm, 1952), these are not likely to be a serious source of

error. I,4-Methyl-histamine, which is present in the brain (S. 1/) has been shown to have 1/350th to 1/200th the activity of histamine on the ileum (Lee and Jones, 1949; Green, 1964; Hye, 1964). β-N-Monomethylhistamine was reported to be more potent than histamine on the ileum (Vartiainen, 1935; Schild, 1947). Other workers, however, have found that the potency is about 80% that of histamine on the same preparation (Lin, 1961; Bertaccini and Vitali, 1964; Adam and Hye, 1966). This histamine derivative, however, has not been identified in brain extracts of the ox (Werle and Palm, 1952) and cat (Adam and Hye, 1966). N,N-Dimethylhistamine which has about 3/4th the activity of histamine on the ileum (Lin, 1961; Bertaccini and Vitali, 1964; Adam and Hye, 1966) has not been found in cat brain (Adam and Hye, 1966).

These results provide evidence that activity assayed in the test solutions is probably due to histamine (Adam, 1961) and not a mixture of histamine and other pharmacologically active substances.

SECTION 3

RESULTS

3/I RECOVERY OF HISTAMINE

The results of recovery experiments are given in Table 8. The amount recovered in each case is shown and is also expressed as a percentage of the amount added. The mean recovery of histamine when 25 to 100 ng of the amine was added to brain tissue samples (Nos. I to I8 in the Table), was 70% (range 30-95). These recoveries are similar to those reported for the dog (Adam, 1961) and cat (Adam and Hye, 1966). When histamine was added to supernatant (Nos. 19 to 24 in the Table), the mean recovery was 97 (range 89-100); when added to buffer solution (Nos. 25 to 47), 91% (range 80-100). When histamine was added to buffer in quantities of 500 and 1000 ng (Nos. 48 and 49 in the Table), none was detected in the effluent which had been applied to a second column; it is therefore unlikely that incomplete adsorption was a cause of loss. When hypothalamic tissue was extracted three times with trichloracetic acid (Hye, 1964), more than 99% of the histamine extractable from the tissue was present in the first extraction. Hence it appears that most of the loss occurs during the extraction

Table 8

Recovery of histamine

Histamine added to portions of rabbit cerebellum (50-60 mg) after precipitation with trichloracetic acid

Experiment number	Histamine added (ng)	Estimated recovery* (ng)	Percentage Recovery (approximately)
1	100	86.1	86
2	100	85.8	86
3	100	95.0	95
4	100	95.3	95
5	50	34.2	68
6	50	34.0	68
7	50	34.1	68
8	50	41.4	83
9	25	8.2	33
10	25	10.0	40
11	25	7.7	31
12	25	6.8	27

Histamine added to 4 ml aliquots of cerebral cortex (equivalent to 60 mg of tissue) after precipitation with trichloracetic and dilution with water

13	100	82.9	83
14	100	91.0	91
15	50	42.4	85
16	50	42.4	85
17	25	11.5	46
18	25	14.0	56

Histamine added to 4.6 ml aliquots of supernatant from cerebellar tissue (equivalent to 60 mg) after precipitation with trichloracetic acid, centrifugation and neutralization

19	100	99.0	99
20	100	95.3	95
21	50	50.0	100
22	50	49.3	98
23	25	22.3	89
24	25	25.0	100

In experiments Nos. 1-24 the values were corrected for the amount present in the control samples, as explained in the text

Table 8 (continued)

Histamine added to buffer solution for absorption: 10 ml buffer, 0.05 molar, 100 mEq/l Na^+, pH 8.0

Experiment number	Histamine added (ng)	Estimated recovery (ng)	Percentage recovery (approximately)
25	100	90	90
26	100	92	92
27	100	90	90
28	100	88	88
29	100	88	88
30	100	90	90
31	100	88	88
32	100	94	94
33	50	46	92
34	50	48	96
35	50	48	96
36	50	44	88
37	50	45	90
38	50	50	100
39	50	44	88
40	50	44	88
41	25	23	92
42	25	24	96
43	25	22	88
44	25	22.5	90
45	25	25	100
46	25	20	80
47	25	22.5	90
48*	1000	1000	100
49*	500	500	100

* In experiments Nos. 51 and 52 no histamine was detected in the effluent.

Summary of Table

Mean percentage recoveries

Histamine added (ng)	100	50	25	Mean recovery ± S.E.
to brain	89 (6)	76 (6)	39 (6)	68 ± 6 (18)
to supernatant	97 (2)	99 (2)	94 (2)	97 ± 2 (6)
to buffer	90 (8)	92 (8)	91 (7)	91 ± 1 (23)

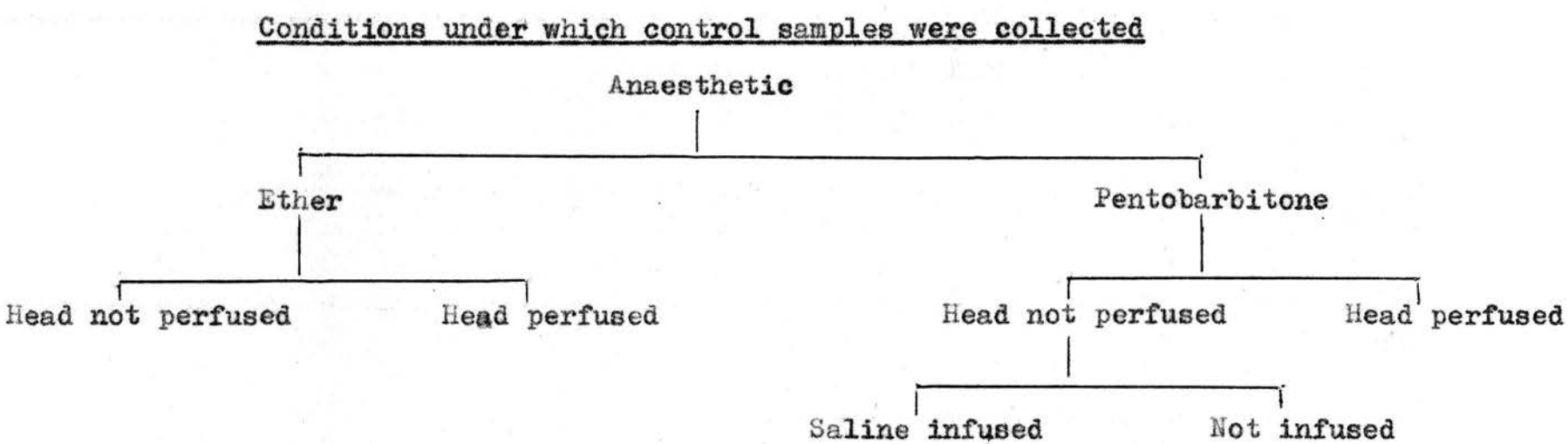
(no. of experiments)

and centrifugation step; that a portion of histamine is destroyed or irreversibly bound to the precipitate.

3/2 DISTRIBUTION OF HISTAMINE IN THE RABBIT BRAIN AND HYPOPHYSIS

Values from different parts of the brain and hypophysis were obtained under various conditions as shown in the Diagram (Table 9, 10, 11). In the earlier experiments rabbits were anaesthetized with ether; later, it was found that the animals were more easily maintained under pentobarbitone anaesthesia. Some brain samples were obtained after perfusing the head with Ringer-Locke solution (S. 2/6); others after i.v. infusion with isotonic saline (16.7 ml/Kg/2 hr).

The results were analysed statistically under each condition and the significance of the difference between means (saline infused v. not infused; head perfused v. not perfused; ether v. pentobarbitone) was determined by "Student's" t test. The comparisons were based on values obtained for the anterior lobe of the hypophysis, the hypothalamus and occasionally for other parts of the brain.


Conditions under which control samples were collected
Anaesthetic
Ether
Pentobarbitone
Head not perfused
Head perfused
Head not perfused
Head perfused
Saline infused
Not infused

3/2/1 Effect of saline infusion

Pentobarbitone anaesthetic

Head not perfused

Saline infused | Not infused

Table 9

Region	Estimates of concentration, as ng/g fresh tissue	
	Saline infused	Not infused (individual results)
Anterior lobe of hypophysis		
Mean	690	650, 480, 350, 740
S.E.	60	
S.D.	200	
Range	500-1250	
Fiducial Limits (P = 0.05)	570-820	
No.	13	
Hypothalamus		
Mean	690	580, 620, 470, 490
S.E.	60	
S.D.	150	
Range	490-910	
Fiducial Limits (P = 0.05)	550-830	
No.	7	

Two out of the four values from the group without saline infusion fell outside the fiducial limits of the group withsaline infusion. In view of the small number in the group, it seemed reasonable to pool the two groups for further comparison.

3/2/2 Effect of anaesthetic

Head not perfused

Ether

Pentobarbitone (combined values with and without saline infusion)

Table 10

Region	Estimates of concentration as ng/g fresh tissue					
	Ether			Pentobarbitone		
	Mean±S.E.	No.	Limits (P 0.05)	Mean±S.E.	No.	Limits (P 0.05)
Anterior lobe of hypophysis	690±100	6	440-940	660±50	17	560-760
Hypothalamus	670±50	6	530-810	640±40	11	540-730

Since the differences between the means for the anterior lobe of hypophysis and for the hypothalamus are not statistically significant ($P > 0.8$), the results of the two groups were pooled for each of the tissues and used in another comparison.

3/2/3 Effect of perfusing the head with Ringer-Locke Solution

Pooled values collected under ether or pentobarbitone anaesthesia

Head not perfused — Head perfused

Table 11

Region	Estimates of concentration as ng/g of fresh tissue					
	Head not perfused			Head perfused		
	Mean±S.E.	No.	Limits (P 0.05)	Mean±S.E.	No.	Limits (P 0.05)
Ant.lobe of hypophysis	670±40	23	580-760	590±60	8	440-730
Hypothalamus	650±30	17	580-720	700±40	8	600-810
Thalamus	250±20	11	210-290	290±30	8	210-370
Central grey matter	300±20	15	260-340	240±20	7	180-300
Cortex	120±10	11	100-140	100±10	12	70-120
Cerebellum (vermis)	60±5	12	50-70	30*±5	9	15-40

*Significantly different, $P < 0.01$

The object of perfusing the head was to estimate the proportion of histamine which could derive from residual blood in the tissue samples.

Sokoloff (1961) used an isotopic method to measure capillary blood content of the cat's brain: the mean content was 1.2% of tissue weight. In the present studies the mean value for the concentration of histamine in rabbit whole blood was about 4 µg/ml (Table 3). On the basis of Sokoloff's findings (1961), it was calculated that blood might contribute 50 ng of histamine per gram of brain tissue. However, the mean values of various samples obtained after perfusion of the head were indistinguishable from those where the animals had only been bled. The exception was the cerebellum, in which the mean concentration fell by 50% after perfusion (from 60 to 30 ng/g). It is therefore probable that about 30 ng of histamine extractable from one gram of brain tissue is blood histamine. However, the values reported in this work have not been corrected for the possible contribution of histamine by blood.

According to Sokoloff (1961), the capillary blood content of brain is relatively uniform.

Hence, were histamine in extracts of brain to derive wholly from blood, its distribution would also be expected to be uniform. Table shows that histamine is, in fact, unevenly distributed in brain. Since perfusion of the head made no significant difference to the mean estimates, it was not adopted as a routine procedure.

3/2/4 Pooled results

Because it was not possible to distinguish between the effects of perfusion, anaesthetic and saline infusion, the results were pooled and re-calculated (Table 12). The concentrations are expressed as nanograms per gram of tissue weight and these have been rounded off to the nearest 10 ng after calculations. The standard error (S.E.) of the mean value is given to the nearest whole number. Where the areas are represented by few rabbits, only the mean value and the range are given.

Table 13 shows the calculated amount of histamine in the hypophysis and in samples of brain.

Hypophysis. Mean values for the concentration of histamine in the anterior lobe (650 ng/g) and posterior lobe (400 ng/g) are lower than those reported for the dog (Adam, 1961) and cat (Adam and Hye,

Table 12

Histamine in rabbit's hypophysis and brain (pooled controls). Estimates of concentration as ng/g of fresh tissue

Region	No. of rabbits	Mean	Range	S.E. of mean	Limits of the mea (P = 0.05)
HYPOPHYSIS					
Anterior lobe	31	650	340-1250	36	570-720
Posterior lobe	15	400	180-580	35	330-480
WHOLE BRAIN	3	130	120-140		
DIENCEPHALON					
Basal ganglia	3	270	200-320		
Hypothalamus	25	660	470-910	27	610-720
Medial thalamus	19	270	140-440	18	230-310
MIDBRAIN	3	190	180-200		
Superior corpus quadrigeminun	10	210	150-300	17	170-250
Inferior corpus quadrigeminun	10	170	100-210	11	150-190
Tegmental region	12	170	110-260	12	140-190
Central grey matter	22	280	160-400	15	250-310
HINDBRAIN	3	80	60-90		
Region of pons and medulla	20	140	60-360	15	110-170
Floor of 4th ventricle	8	180	110-360	28	110-250
CEREBRUM	4	120	90-170		
Cerebral cortex	23	110	50-170	7	90-120
Caudate nucleus	12	150	90-220	12	120-180
Hippocampus	10	90	50-150	8	70-110
CEREBELLUM	4	80	60-90		
Vermis	12	60	30-90	5	50-70
CHOROID PLEXUS	8	610	30-1800		

Table 13

Weight of tissue samples and estimates of the amount of histamine contained (number of rabbits same as in Table12)

Region	Mean weight (mg)	Range (mg)	Mean histamine content (ng)	Conc. in tissue (ng/mg)	Range (ng)
HYPOPHYSIS					
Anterior lobe	21.6	11-50	14	2·8	8-27
Posterior lobe	5.5	3-8.5	2.2	0·44	1.0-3.2
WHOLE BRAIN	9700	8200-12900	1260		1160-1360
DIENCEPHALON					
Basal ganglia	1000	830-1170	270		200-320
Hypothalamus	50	23-71	33	6·6	23-45
Medial thalamus	45	29-92	12	2·4	6-20
MIDBRAIN	700	590-840	133		126-140
Superior corpus quadrigeminun	69	54-100	12	2·4	9-18
Inferior corpus quadrigeminun	46	22-68	8	1·6	5-10
Tegmental region	58	41-72	10	2	6-15
Central grey matter	59	32-92	17	3·4	10-24
HINDBRAIN	900	810-920	72		54-81
Region of pons and medulla	68	46-88	10	2·0	4-25
Floor of 4th ventricle	64	44-79	11	2·25	7-23
CEREBRUM	2600	2300-2900	312		234-442
Cerebral cortex	66	37-92	7	1·4	3-11
Caudate nucleus	70	33-90	10	2·0	6-15
Hippocampus	67	40-95	6	1·2	3-10
CEREBELLUM	1250	1100-1400	100	25	75-112
Vermis	59	45-90	4	0·8	2-5

1966) and varied over a narrower range. Occasionally high concentrations were encountered in the anterior lobe. The weight of the posterior lobe was small and variable and occasionally contained no detectable quantities of histamine could be detected by the method. The concentration of the amine was higher in the anterior lobe than in the posterior lobe.

Hypothalamus. The highest concentration in the brain was in the hypothalamus (mean 660 ng/g). Estimates for the hypothalamus and for most parts of the brain were less variable than those for the hypophysis. Beyond the hypothalamus, the concentration fell progressively and did not exceed 280 ng/g.

Rest of the brain. In the medial thalamus the mean concentration was 270 ng/g. In 4 parts of the midbrain, the mean concentration was in the range 170 to 280 ng/g. Elsewhere, the concentration was less than 180 ng/g, being lowest in the cerebellum (60 ng/g). Thus the difference in concentration between the hypothalamus and cerebellum was about 10-fold.

3/3 ESTIMATES OF HISTAMINE IN THE RABBIT WHOLE BLOOD

Collection of blood samples for the estimation of histamine has been described in Section (2/1). 0.05 ml of blood was taken and histamine was extracted in the manner similar to the extraction and purification of histamine from brain tissues.

Saline infusion experiments were conducted as controls for the intravenous infusion of amino acids. One blood sample was collected before the infusion of saline and another just before the end of infusion or before bleeding. Blood samples were also taken for the haematocrit, before and after infusion. The concentration of histamine in blood after infusion was corrected for the change in haematocrit. Estimates are given in Table 14. Values are expressed as nanograms per ml of blood and these have been rounded off to the nearest 100 ng after calculations. The results show that infusion of saline did not alter significantly the concentration of histamine in blood.

Table 15 shows the mean concentration of histamine in rabbit whole blood from 127 untreated animals.

Table 14

Estimates of concentration of histamine in rabbit whole blood, before and after intravenous infusion of saline

Rabbit No.	Estimates of concentration as ng/ml		
	Before infusion	After infusion	Corrected values
1	3800 (42)	3300 (38)	3600
2	2200 (33)	2700 (33)	2700
3	2700 (46)	2700 (45)	2800
4	3000 (39)	2700 (39)	2700
5	2700 (42)	2500 (40)	2600
6	3300 (40)	3200 (39)	3300
7	7000 (35)	7600 (34)	7700
8	4300 (39)	3700 (37)	3900
9	6500 (33)	4900 (30)	5400
10	6500 (42)	6500 (42)	6500
11	2700 (42)	2700 (42)	2700
Rabbits 1-11 Mean±S.E.	4060±530	3900	4000±530
Haematocrit	(39.5)	(38)	

( ) Haematocrit value: per cent of Packed Cell Volume.

In rabbit 1 and 2 (After infusion), samples were taken before bleeding; the rest were taken just before the end of infusion.

Table 15

Estimates of the concentration of histamine in whole blood of untreated rabbits (as ng/ml)

No.	127
Mean	3900
S.D.	1400
S.E.	130
Fiducial Limits (P 0.05)	3600-4100
Range	1600-8800

SECTION 4

DISCUSSION

References has already been made to earlier work on histamine in rabbit brain (Table 1) and in whole blood (Table 3). Values have not so far been reported for the hypophysis.

The object of the present work was to prepare a detailed map of the distribution of histamine in the rabbit's brain and hypophysis, thereby relating the anatomical area or region to the concentration of the amine.

The method of estimating histamine in brain tissue was that of Adam (1961). The recoveries

by this method were consistent in the range 25 to 100 ng of histamine added to brain tissue (Table 8). Various tests of specificity (Adam, 1961; Adam and Hye, 1966) have provided convincing evidence that the activity estimated in the assay is probably histamine.

4/1 BLOOD

The mean estimate was 3.9 µg/ml with range 1.6 to 8.8 (127) and is comparable with values reported by other workers (Table 3). The correlation coefficient (r) was calculated to test for dependance between the concentration of histamine in blood and the quantity of amine extractable from hypophysis or brain. Table 16 gives the value of 'r' and the probability of its significance ('Student's' t test). The tests show that there is no significant positive correlation between the two variables, although in some instances there is a positive trend. These findings suggest that most of the histamine extractable from brain and hypophysis is derived from the actual tissue and not from blood.

4/2 HYPOPHYSIS

The concentration of histamine in the anterior and posterior lobes of the hypophysis is lower than

Table 16

Test of correlation between the concentration of histamine in whole blood and the concentration of the amine extractable from hypophysis or brain

Region	r	Probability of significance
Anterior lobe of hypophysis	+ 0.53	> 0.80
Tegmental region	- 0.09	> 0.80
Central grey matter	+ 0.21	> 0.70
Inferior corpus quadrigeminun	- 0.24	> 0.70
Region of Pons and medulla	- 0.24	> 0.60
Caudate nucleus	+ 0.34	> 0.40
Hippocampus	+ 0.53	> 0.30

that reported for the dog (Adam, 1961) and cat (Adam and Hye, 1966) and less variable (Table 2). In the dog and cat the high concentration of histamine in the gland is associated with the presence of mast cells (S. 1/3).

It is not yet known whether the rabbit's hypophysis contains mast cells. The lower concentration of histamine in the gland and the paucity of mast cells in the rabbit's organs and tissues (Constantinides, 1953) suggest that hypophysial histamine does not derive from mast cells. Indirect evidence presented in Part II of this thesis, on the effect of reserpine, favours this view; experiments with reserpine showed that in the anterior lobe of the hypophysis histamine was depleted to the same extent as in the hypothalamus. Since reserpine does not release histamine from mast cells (Parrat and West, 1957), it can be assumed that histamine in the hypophysis is probably not in mast cells. Histological studies are needed to test this assumption.

4/3 BRAIN

Origin of brain histamine. In theory, histamine in brain may derive from (a) mast cells, (b) the blood (platelets) and (c) neural tissue.

(a) Mast cells. As already mentioned (S. 1/3) mast cells have not been found in the brain of several mammalian species, including the rabbit (Constantinidès, 1953). Studies on the subcellular distribution of histamine in the brain of rat and dog indicate that the amine is not present in mast cells (Carlini and Green, 1963; Michaelson and Dow 1963; Katoka and Robertis, 1967). So far, studies are not available on the subcellular localization of histamine in the rabbit brain.

(b) Blood. The evidence supporting the assumption that histamine extractable from brain or hypophysis is mostly tissue amine and not derived from blood is summarized below:

(1) Perfusion of the head with Ringer-Locke solution (S. 3/2/3) did not produce a detectable fall in the concentration of histamine in hypophysis or brain (except in the cerebellum).

(2) Histamine is unevenly distributed in the brain (S. 3/2/3).

(3) The concentration of histamine in blood is not correlated with that of the brain or hypophysis (Table 16).

Further evidence is presented later in the thesis.

Distribution. The distribution of histamine in rabbit brain is similar to that found in the dog (Adam, 1961) and cat (Adam and Hye, 1966), though the concentrations in the hypothalamus and medial thalamus are lower. The present results do not agree with the high values reported for the rabbit (Shore, Burkhalter and Cohn, 1959; Waalkes, Coburn and Terry 1959, See Table 1). These authors estimated histamine by chemical methods and concluded that it was more or less uniformly distributed in the brain.

In the present study, the highest concentration w as found in the hypothalamus followed by the medial thalamus, midbrain, pons-medulla, cerebral cortex and cerebellum. The gradient across these regions is difficult to explain but might be related to morphological factors such as density of neural cells or the distribution of histidine DC and the methylating enzyme.

Comparison of histamine distribution with DC and INMT activities. The high histamine concentration in the hypothalamus agrees well with the finding that the hypothalamus in the cat has a high

capacity to form histamine from histidine (S.1/6) (White, 1959, 1960). Parts of the brain in the cat, like the cerebral cortex and cerebellum which show little capacity to form histamine *in vitro* (White, 1959) also contain very little histamine.

Brain tissue catabolizes histamine by ring methylation (Brown et al, 1959) and the methylating enzyme is present in most parts of the brain (Axelrod et al, 1961, See S.1/7). The distribution of histamine in the rabbit brain does not seem to parallel the distribution of INMT activity. According to Brown et al (1959) the enzyme activity is more or less evenly distributed in the rabbit brain.

Comparison with monoamines. The distribution of noradrenaline and dopamine in the rabbit brain has been studied in some detail by Matsuoka, Yoshida and Imaizumi (1964), using chemical methods (Table 17). The mean concentrations of noradrenaline and dopamine in the hypothalamus were 810±330 and 470±220 (S.E.) ng/g respectively, and are roughly comparable with the mean concentration of histamine, (660±27 ng/g). Table 17 shows the distribution of catecholamines in the rabbit brain as reported by

Table 17

Regional distribution of some biogenic amines in the rabbit brain. Estimates, as ng/g.

	Whole brain	Brain-stem	Hypothalamus	Thalamus	Midbrain	Pons-Medulla	Cerebral cortex	Cerebellum	Caudate nucleus
Histamine (Present work) No. of values	130 3		660±27 25	270±18 19	170-280* 54	140±15 20	110±7 23	60±5 12	150±10 12
Noradrenaline		380±22 (8) 431±39 (8) 540-650† (13) 550-620† (10) 510-620† (5)	810±330 (14) 1090±70 (12)	220±80 (14)	320 (6)	50-80* (14) 260 (6)	80±30 (14) 116±6 (12)	60±0.0 (14)	850±340 (14)
Dopamine		122±29 (12)	352±92 (12) 470±220 (14)	270±50 (14)		180-320* (14)	270±120 (14) 265±31 (12)	340±160 (14)	8600±2600 (13) 4280±740 (14)
5-Hydroxy-tryptamine	570±80 (2) 460±30 (1) 500 (7)	620-740† (5) 670 (9) 590-710† (13)	430±120 (11)		830±40 (3) 647±38 (4) 663 (6) 378±22 (8)	630±50 (3) 582±38 (4) 582 (6)	55±2 (11) 282±32 (4) (telencephalon)	120±10 (3) 112±11 (4)	

All the values reported for catecholamines in this table were assayed by chemical methods. The values for 5-HT were assayed chemically, except those reported by Costa and Colleagues, which were estimated biologically. Costa et al (1960) perfused the rabbit head with saline.

( ) reference

* range of concentrations in different parts

† range of several means.

References

(1) Bogdanski, Pletscher, Brodie and Udenfriend, 1956.
(2) Brodie, Shore and Pletscher, 1956.
(3) Costa and Aprison, 1958.
(4) Costa and Rinaldi, 1958.
(5) Brodie, Spector and Shore, 1959.
(6) Himwich, Costa, Pscheidt, and van Meter, 1959.
(7) Waalkes, Coburn and Terry, 1959.
(8) Costa, Pscheidt, van Meter and Himwich, 1960.
(9) Gursey and Olson, 1960.
(10) Spector, Kuntzman, Shore and Brodie, 1960.
(11) Joyce, 1962.
(12) Weil-Malherbe, Posner and Waldrop, 1962.
(13) Spector, 1963.
(14) Matsuoka, Yoshida and Imaizumi, 1964.

various authors, and the values are compared with the histamine concentrations obtained in the present study. Like histamine, the concentration of catecholamines was highest in the hypothalamus and fell progressively in the thalamus, midbrain, cerebral cortex, hindbrain and cerebellum. In the caudate nucleus the concentration of histamine is much lower than that of catecholamines as reported by Matsuoka et al (1964) and Spector (1963).

Values for 5-HT in various areas of the rabbit brain have been reported by several workers and are shown in Table 17 . The mean concentrations in the hypothalamus is 430±120 (S.E.) ng/g (Joyce, 1962). Values obtained for the midbrain (380-830 ng/g) and the hindbrain (580-630 ng/g) are higher than those found in the present work for histamine (170-280 ng/g for midbrain and 140±15 ng/g for hindbrain). 5-HT, like histamine, is present in large quantities in the rabbit platelets (Table 4). The control values for 5-HT in the rabbit brain stem reported by Costa, van Meter and Himwich (1960) are lower than those obtained by Brodie, Spector and Shore (1959) and others (Table 17), because

"the perfusion of the brain in situ with saline probably removed a portion of the biogenic amine present in the vascular system of the brain" (Costa et al, 1960). These authors, however, made no comparison with values obtained without perfusion; hence the difference might have been due to the different methods employed: Costa et al (1960) assayed 5-HT biologically; Brodie et al (1959) used a chemical method.

Concluding remarks. Like monoamines, histamine is unevenly distributed in the brain which "suggests that the agent has a role to play in the specialized function of those regions where its concentration is high", (Vogt, 1959, S.1/11). Histamine has been isolated from small nerve endings and synaptic vesicles (Katoka and Robertis, 1967). Besides its actions on peripheral organs, it has pharmacological actions on the brain (S.1/11). These facts would seem to support the hypothesis that histamine has a physiological function in the brain. But "until more is known about the exact site of its formation and storage, and the conditions necessary for its release, speculation is likely to be unprofitable" (Adam, 1961). A closer analysis of the effects of

histamine on brain functions is necessary. Study of the possible means of increasing or decreasing the histamine content, and of its formation and catabolism in the brain will shed more light on its functional significance.

PART II

CHANGES PRODUCED BY TREATMENT WITH AMINO ACIDS AND DRUGS

PART II

INTRODUCTION

"Little is known about the effect of drugs on the histamine content of the brain in mammals. This is in sharp contrast to the wealth of information available on the effect of drugs on the levels of serotonin and catecholamines", (Kahlson and Rosengren, 1965). It seemed desirable, therefore, to test whether treatment with certain amino acids and drugs could alter the concentration of histamine in rabbit's brain. Drugs and amino acids were chosen for their known effects on the concentration of monoamines and on the catabolism of histamine. Injection of DOPA or 5-HTP is known to raise the concentration of the corresponding amines in rabbit brain (S. 6). Hence it was of interest to study the effect of histidine on the concentration of histamine in brain and the interaction of histidine with drugs, particularly reserpine.

The major pathway of histamine catabolism in brain is by ring methylation (S. 1/7). The various metabolic steps are shown schematically in Fig. 5. Theoretically, a drug can affect

synthesis, uptake, release or catabolism of histamine in brain, and thus may influence directly or indirectly the concentration of the amine. Hence, the concentration of histamine in brain would be expected to rise after treatment with histidine or with drugs that inhibit catabolism, and to fall after treatment with drugs that interfere with synthesis or storage.

The compounds tested can be conveniently grouped as follows:

(a) Amino acids

1. L-Histidine
2. L-Alpha-methyldopa
3. L-DOPA
4. DL-5-Hydroxytryptophan
5. L-Tryptophan

(b) Drugs

1. Chlorpromazine-hydrochloride
2. Iproniazid-phosphate
3. Reserpine

Various authors have attempted to study the effect of drugs on the concentration of histamine in whole brain of rabbit (Waalkes, Coburn and Terry, 1959; Burkhalter, Cohn and Shore, 1960),

guinea pig (Waalkes et al, 1959) or rat (Ungar and Witten, 1963; Walaszek and Chapman, 1963). Adam and Hye (1966), however, studied the effect of drugs on a regional basis and thus avoided the inclusion of large areas of brain which contain only minute quantities of histamine.

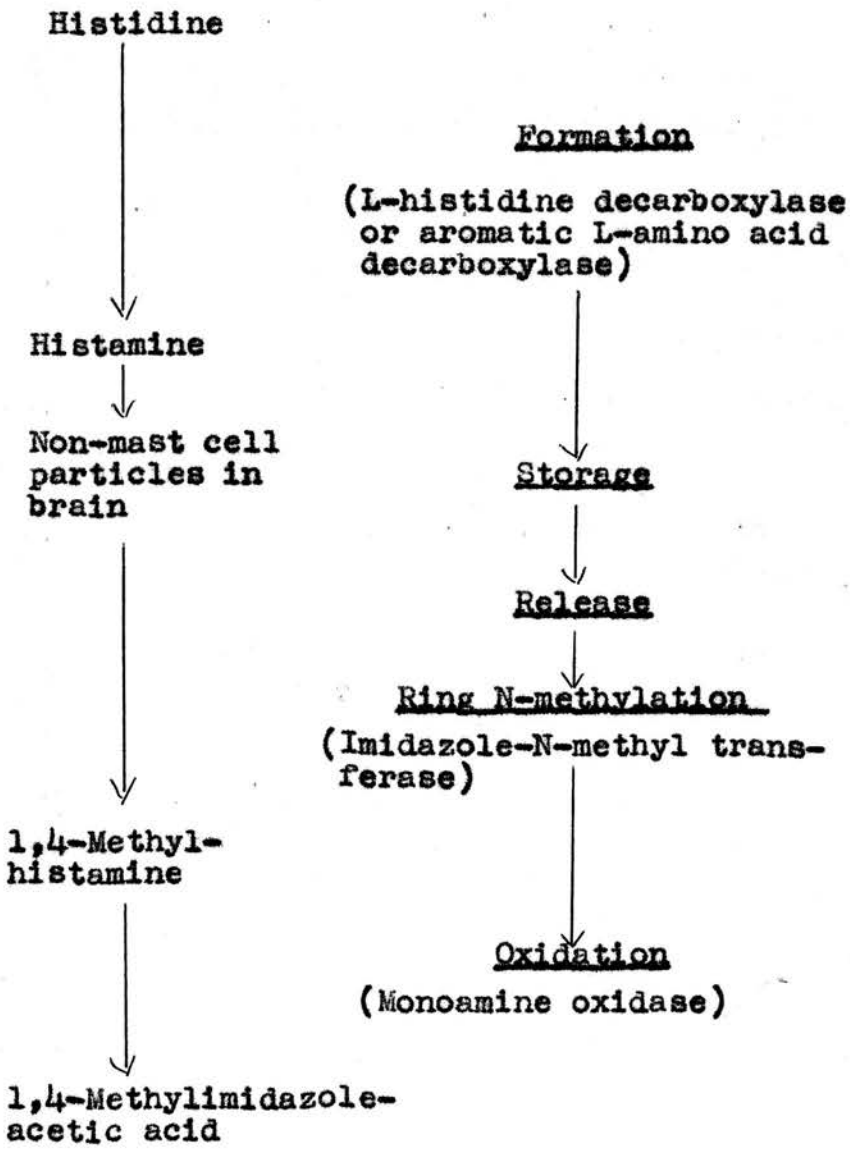
In the present work, various areas of brain were selected for these studies. The anterior lobe of the hypophysis was also included, since it served as a control area outside the blood brain barrier. Blood samples were taken before and after treatment because changes in the concentration of histamine in blood might have contributed to changes observed in the brain.

The extracts were purified and histamine was estimated as described (S. 2/9 and 2/10). In some experiments saline was infused as a simultaneous control for the infusion of amino acids; these values were pooled with those of the untreated controls (Table 12). The significance of the difference between the mean of the control and the mean of the treated group was calculated by 'Student's' t test.

The amino acids and drugs, their administrat-

ion and the results obtained after treatments are described; the effects of the compounds are then summarized and discussed.

Fig. 5. Schematic diagram of pathways for storage and metabolism of histamine in the brain (based on Schayer, 1959). For details, see S.1/3,1/6 and 1/7.



SECTION 5

FORMATION AND UPTAKE OF HISTAMINE

The formation and uptake of histamine have been briefly reviewed (S. 1/6 and 1/8).

In the following experiments the formation of histamine in brain was studied in the conscious rabbit, but the results could not be easily interpreted without further tests. These included:

(a) Perfusion of the head with Ringer-Locke solution.

(b) Infusion of hypertonic solution

(c) Intravenous injection of glycogen

(d) Infusion of histamine

(e) Study of the effect of time on the concentration of histamine in brain after histidine infusion.

(f) Infusion of histidine in the anaesthetized rabbit

(g) Formation of histamine from histidine during the procedure for extraction and purification.

Some of the above experiments are described in this Section; others in the Appendix.

5/1 INFUSION OF HISTIDINE IN THE CONSCIOUS RABBIT

5/1/1 Materials and method

The rabbit was restrained by placing it in a wooden box the top of which was closed by a sliding perspex lid. The front part of the box was cut away so as to allow the head to protrude. Blood samples for haematocrit and histamine estimation were taken before and towards the end of infusion. The preparation of histidine solutions and the procedure for their i.v. infusion has already been described in Section 2/3 and 2/4. Each dose of histidine was infused over a period of about 2 hr. Half an hour after the end of infusion the animal was anaesthetized with pentobarbitone. The carotid arteries were exposed and cannulated; the rabbit was then heparinized and bled; the brain and hypophysis were removed immediately and dissected as described in Section 2/7 and 2/8.

Effect of repeated infusions of histidine.

Rabbits were given 3 infusions spaced over a period of 24 hours. In each infusion the animal received 500 mg/Kg/2 hr.

Two rabbits received a total of 1000 mg/Kg/4 hr.

Effect of a single infusion of histidine.

The doses were 500, 250, 125, 62,30, 15 and 5 mg/Kg/2hr.

5/1/2 Results

The rabbits appeared to be unaffected by the infusion of histidine, showing neither sedation nor excitement nor changes in the rectal temperature.

Effect on histamine concentration. Values for the concentration of histamine in blood, hypophysis and various parts of the brain after different doses of histidine are shown in Table 18. The dose-response relationship is also shown graphically in Fig. 6.

In the dose range 62 to 1500 mg/Kg, histidine increased the histamine concentration in various parts of the brain, but not in the anterior lobe of the hypophysis nor in blood. The concentration rose in the posterior lobe of hypophysis after a dose of 500, 1000 and 1500 mg/Kg. Although the concentrations were highest in the hypothalamus, the greatest percentage rise occurred in the central grey matter and tegmental region of the

Table 18

Effect of histidine infusions on the concentration of histamine in hypophysis, brain and blood of the rabbit.

Mean estimate ± S.E. expressed as nanograms per gram of fresh tissue or nanograms per ml of whole blood, (No. of rabbits).

Region	Control	Dose of histidine (mg/Kg) infused over 2 hours								
		3 x 500 (over 24 hr.)	2x500	500	250	125	62	30	15	5
HYPOPHYSIS										
Anterior lobe	650±36 (31)	720±70 (6)	1400 (2)	800±80 (11)	830±120 (5)	610 (2)	880 (4)	750 (2)	1600 (2)	750 (1)
Posterior lobe	400±35 (15)	760±130 (6)	730 (2)	860±90 (5)						
DIENCEPHALON										
Hypothalamus	660±27 (25)	2310±120 (5)	1600 (2)	1480±130 (5)	1360±120 (5)	950 (2)	1070 (4)	960 (2)	930 (2)	630 (1)
Medial thalamus	270±18 (19)	680±40 (6)	770 (2)	560±70 (5)						
MIDBRAIN										
Superior corpus quadrigeminun	210±17 (10)	770 (3)		440±30 (5)						
Inferior corpus quadrigeminun	170±11 (10)	500 (2)		440±30 (5)						
Reg. Tegmentum	170±12 (12)	640 (2)		440±30 (5)	460±20 (5)	370 (2)	320 (4)	260 (2)	360 (2)	250 (1)
Central grey matter	280±15 (22)	1160±50 (5)		960±70 (6)	800±70 (5)	540 (2)	520 (4)	500 (2)	480 (2)	420 (1)
HINDBRAIN										
Reg. pons and medulla	140±15 (20)	290 (2)		260±30 (5)	280±20 (5)	240 (2)	200 (4)	160 (2)	250 (2)	170 (1)
Floor of 4th ventricle	180±28 (8)	580 (2)		440±90 (5)						
CEREBRUM										
cerebral cortex	110±7 (23)	270±10 (5)	250 (2)	270±20 (5)						
Caudate nucleus	150±12 (12)	280±40 (5)		330±30 (5)	290±20 (5)	260 (2)	250 (4)	200 (2)	190 (2)	170 (1)
Hippocampus	90±8 (10)	310 (3)		170±20 (5)						
CEREBELLUM										
vermis	60±5 (12)	110 (3)	80 (21)	130±30 (5)						
BLOOD before infusion	4060±530 (11)	3500±380 (5)	2900 (2)	3300±400 (10)	3800±700 (5)	3600 (2)	4000 (4)	5200 (2)	6200 (2)	4300 (1)
BLOOD towards end of infusion*	4000±530 (11) (saline infusion)	4200±340† (5)	2400 (2)	2900±500 (10)	3700±800 (5)	3400 (2)	4100 (4)	5200 (2)	6400 (2)	4300 (1)

* † Values corrected for changes in the haematocrit reading
Blood samples taken just before bleeding the rabbits

In the dose range 250 to 3x500 mg/Kg histidine the increases in different parts of the brain were highly significant ($P < 0.01 - < 0.001$).

midbrain. In the hypothalamus, the mean percentage increase over the control was from 50 to 250%; in the central grey matter, from 90 to 320%; in the tegmental region, from 90 to 280%; in the pons-medulla, from 50 to 110% and in the caudate nucleus, from 70 to 120%. After a dose of 500 mg/Kg, the concentration of histamine also rose significantly in other parts of the brain, namely, medial thalamus, superior and inferior corpora quadrigemina, cerebral cortex, hippocampus, floor of 4th ventricle and cerebellum; the increases in these regions were in the range 90 to 160%. In the dose range 62 to 1500 mg/Kg the increases were in most cases highly significant ($P < 0.01$ - < 0.001). Where the number of values available were to 2 to 4, they were found to fall outside the upper limits of the controls ($P = 0.05$).

Within the dose range 5 to 30 mg/Kg, 5 animals were used. Only in the midbrain (central grey matter and tegmental region) did the results fall outside the fiducial limits of the control.

5/2 INFUSION OF HISTAMINE

Since histamine may have been formed outside the brain during the infusion of histidine, control

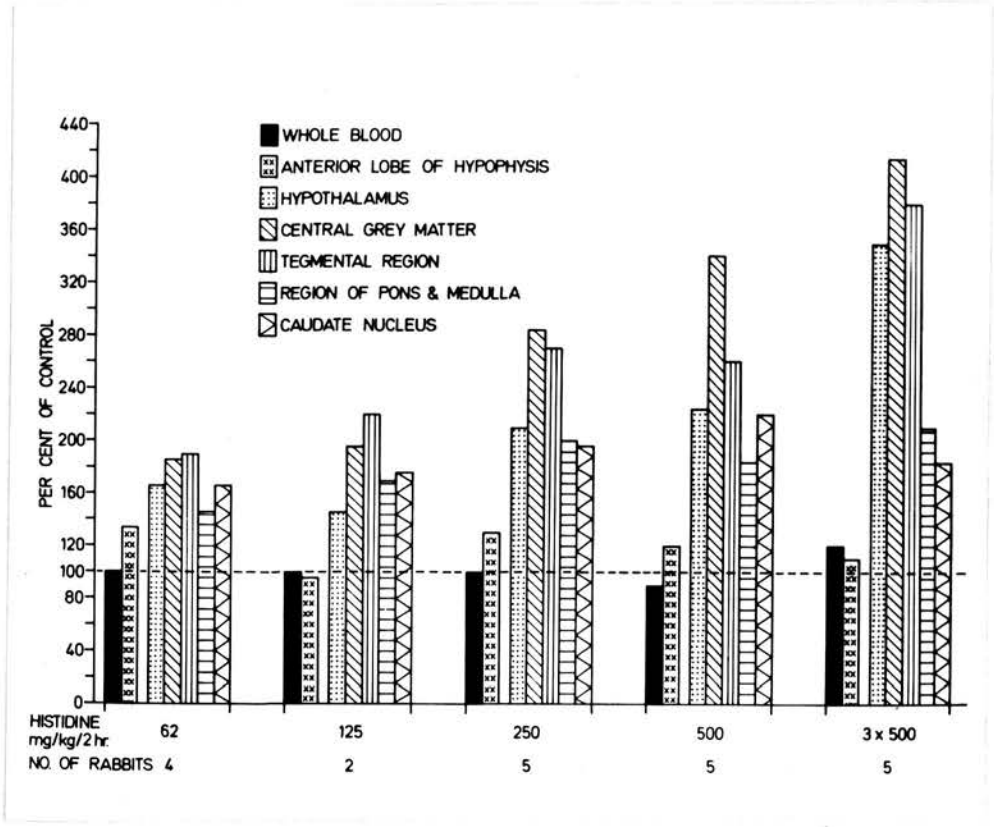


Fig. 6. Effect of i.v. infusions of histidine on on the concentration of histamine in blood, hypophysis and brain. The horizontal line at 100 per cent represents the mean control value for the concentration of histamine. The columns represent the mean concentrations of histamine expressed as percentages of the control after various doses of histidine. Each dose was infused at a constant rate over a period of about 2 hr. The rabbits were anaesthetized 30 min. after the end of infusion and then sacrificed. The last group received 3 separate infusions of histidine (500 mg/Kg/2 hr) over a period of 24 hr.

experiments were performed to test for the possible entry of histamine from blood into the brain.

5/2/1 Materials and method

Rabbits received 5 mg histamine base per Kg body weight. The total amount was dissolved in 30 ml of sterile isotonic saline and infused i.v. over a period of about 2 hr. The slow injector delivered 0.25 ml/min., hence the rate of histamine infusion was about 42 µg/Kg/min. Thirty minutes after the end of infusion, rabbits were anaesthetized and bled. Histamine was estimated in both lobes of the hypophysis, whole blood and various regions of the brain. Five rabbits were used. In rabbits 2, 3, 4 and 5 (Table 19) the head was perfused with Ringer-Locke solution.

5/2/2 Results

Rabbits were observed for gross pharmacological signs throughout the infusion. About 30 min. after the start of infusion the breathing became rapid and laboured and eventually the animal was prostrated. Other signs included reddening of the ears and eyes, relaxation of the nictitating membrane, proptosis, profuse salivation, lacrimation and running nostrils.

Table 19

Effect of intravenous infusion of histamine (5 mg/kg/ 2 hr) on the concentration of histamine in hypophysis, brain and blood. Head perfused with Ringer-Locke solution in rabbits No. 2, 3, 4 and 5.

	Estimates expressed as ng/g of fresh tissue or ng/ml of whole blood							
	Anterior lobe of hypophysis	Posterior lobe of hypophysis	Hypothalamus	Medial thalamus	Cerebral cortex	Cerebellum (vermis)	Whole blood	
							Pre-infusion	Post-[+] infusion
Rabbit 1	5130	3510	770	410	210	120		
Rabbit 2	5440	3040	1010	370	180	90		
Rabbit 3	5440	4800	800	310	180	70	2600	3000
Rabbit 4	5580	3720	460	190	140	40	3300	4100
Rabbit 5	3590	1630	410	180	70	40	3500	4400
Rabbit 1 - 5 Mean ± S.E.	5040 ± 370[*]	3340 ± 490[*]	690 ± 110	290 ± 50	160 ± 20[*]	70 ± 20[*]	3100	3800
Control Mean ± S.E. (No.)	650 ± 36 (31)	400 ± 35 (15)	660 ± 27 (25)	270 ± 18 (19)	110 ± 7 (23)	30 ± 6[■] (9)	4060 ± 530 (11)	4000 ± 530 (11)

[*]Significantly different from control P <0.001 (anterior and posterior lobes of hypophysis),
P <0.05 (cerebral cortex),
P <0.01 (cerebellum).

[+]Blood samples taken 8 and 4 min. after the end of infusion, and 4 min. before the end of infusion respectively. These values all corrected for changes in the haematocrit reading.

[■]Values obtained after head perfusion.

The results (Table 19 and Fig. 7) show that the anterior and posterior lobes of the hypophysis take up histamine from the circulating blood. In each, the concentration rose by about 700%. In the hypothalamus and thalamus, histamine concentration did not rise detectably; but in the cerebral cortex and cerebellum an increase of 50 and 130% respectively, was noted. Blood samples taken few minutes before or after the end of infusion, showed only a slight increase.

5/3 EFFECT OF TIME ON THE CONCENTRATION OF HISTAMINE AFTER THE INFUSION OF HISTIDINE

In theory, the newly-formed histamine might be in the cell cytoplasm or stored in granules; if in the cytoplasm, it would be expected to disappear rapidly by catabolism; if in the granules, the turnover might be slower. The concentration of histamine in brain was therefore estimated at different intervals of time after completing the infusion of histidine.

5/3/1 Method

At the end of infusion, the animals were anaesthetized and killed at 16 and 32 hr. Rabbits received 500, 250 and 62 mg/Kg/2hr. Eight animals were used.

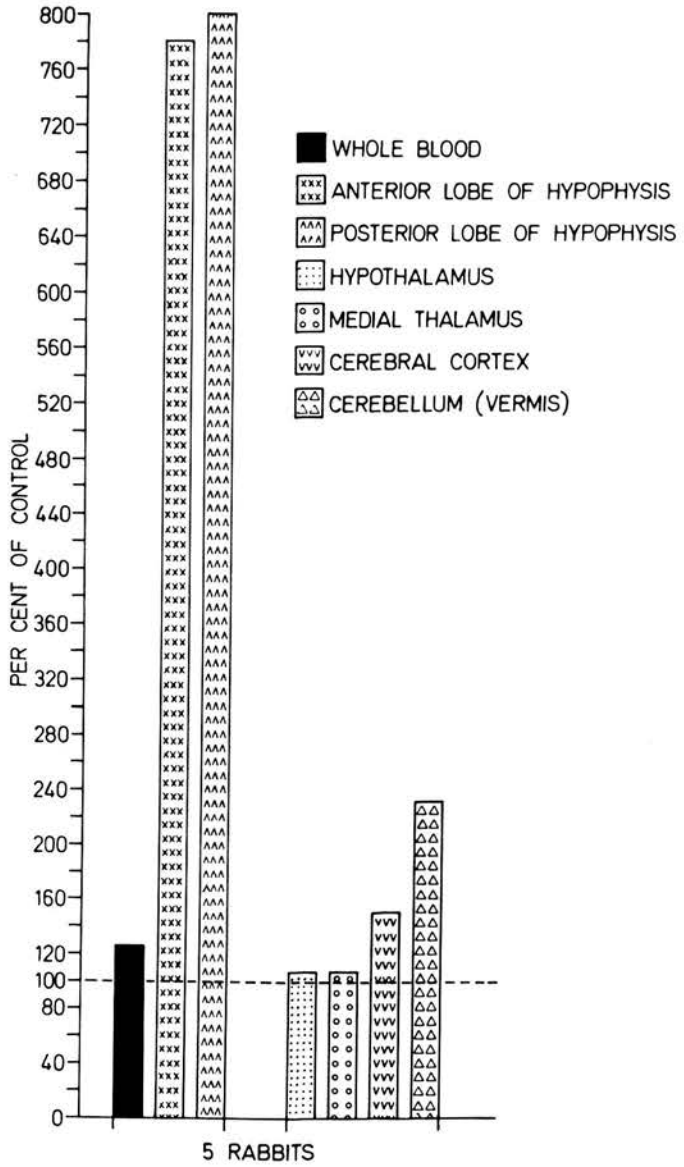


Fig. 7. Effect of intravenous infusion of histamine (5 mg/Kg over 2 hr) on the concentration of histamine in whole blood, anterior and posterior lobes of the hypophysis and different regions of the brain, expressed as a percentage of the control. Rabbits were anaesthetized 30 min after the end of infusion and then sacrificed.

5/3/2 Results

Table 20 shows the concentration of histamine in blood, hypophysis and various parts of the brain at 16 and 32 hr after the infusion. These times indicate the interval between the end of infusion and the start of anaesthesia. The results are compared with the effect of similar doses of histidine at ½ hr. The means and S.E. of the untreated controls are also included in the Table for comparison. Results are also shown in Fig. 8.

Sixteen hr after the infusion of 500 mg/Kg, the concentration of histamine in different regions of the brain was still as high as at ½ hr.

Sixteen hr after the infusion of 250 mg/Kg, the concentration of histamine in the midbrain, hindbrain and caudate nucleus was slightly lower than that at ½ hr; in the hypothalamus, however, the values were comparable with those of the untreated controls. At 32 hr the concentration of histamine in all areas of the brain examined lay within the limits of the means of the untreated controls.

Table 20

Effect of time on the concentration of histamine in hypophysis, brain and blood after various doses of histidine

Time interval indicates the number of hours between the end of infusion and the start of anaesthetic
Mean estimate ± S.E. expressed as nanograms per gram of fresh tissue or nanograms per ml of whole blood (No. of rabbits).

	Dose of histidine mg/Kg/2hr.	Time Interval (hour)	Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Tegmental region	Reg. pons and medulla	Caudate nucleus	Blood before infusion	Blood towards end of infusion
Untreated Controls			650±36 (31)	660±27 (25)	280±15 (22)	170±12 (12)	140±15 (20)	150±12 (12)	4060±530 (11)	4000±530 (11)
Rabbit 1	500	16	380	1290	830	450	300	300	2700	2700*
Rabbit 2	500	16	630	1690					4100	4500*
Rabbit 3	500	16	610	1040					2200	2200*
Histidine Controls	500†	½	800±80 (11)	1480±130 (5)	960±70 (6)	440±30 (5)	260±30 (5)	330±30 (5)	3300±400 (10)	2900±500 (10)
Rabbit 4	250	16	710	780	600	320	270	240	4100	3500
Rabbit 5	250	16	920	980	460	370	200	180	2200	2300
Rabbit 6	250	32	690	550	280	180	100	130	4100	4200
Rabbit 7	250	32	490	500	300	170	130	110	3300	3200
Histidine Controls	250†	½	830±120 (5)	1360±120 (5)	800±70 (5)	460±20 (5)	280±20 (5)	290±20 (5)	3800±700 (5)	3700±800 (5)
Rabbit 8	62	16	950	600	320	140	100	110	3800	3400
Histidine Controls	62†	½	880 (4)	1070 (4)	520 (4)	320 (4)	200 (4)	250 (4)	4000 (4)	4100 (4)

* taken 16 hr. after end of infusion
† values taken from Table 18 , page 100a.

Sixteen hr after the infusion of 62 mg/Kg, the values of histamine were not different from those of the untreated controls.

Within this dose range and at these time intervals, the concentration of histamine in blood and anterior lobe of the hypophysis remained unchanged.

5/4 INFUSION OF HISTIDINE IN THE ANAESTHETIZED RABBIT

Infusion of a large dose of histidine (500 mg/Kg/2hr) in the anaesthetized cat (Appendix 1) did not produce a significant rise of histamine in the hypothalamus or cerebral cortex. The failure might be explained in several ways, including the effect of the anaesthetic.

5/4/1 Materials and method

The procedure was similar to that used in Section 5/1 and described in Section 2/3 and 2/4 except that rabbits were first anaesthetized with pentobarbitone and then infused with various doses of histidine (500, 250 and 62 mg/Kg/2hr). Histamine was estimated in whole blood, anterior lobe of the hypophysis and various parts of the brain. Six rabbits were used.

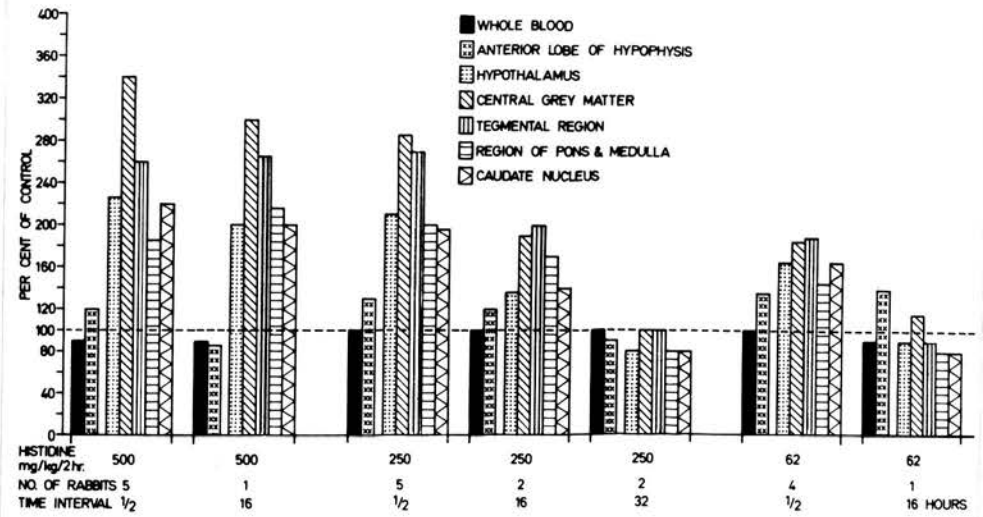


Fig. 8. Effect of time on the concentration of histamine in blood, hypophysis and brain after various doses of histidine. The horizontal line at 100 per cent represents the mean control value for the concentration of histamine. The columns represent the mean concentrations of histamine expressed as percentage of the control in whole blood, anterior lobe of the hypophysis and 5 regions of the brain, after intravenous infusions of various doses of histidine (500, 250 and 62 mg/Kg/2 hr). Rabbits were anaesthetized at ½, 16 or 32 hr after the end of infusion and then sacrificed.

5/4/2 Results

These are presented in Table 21. In the dose range 62-500 mg/Kg, there was no evidence of an increase in the concentration of histamine in blood or anterior lobe of the hypophysis, with the exception of rabbit (3), where the value for the anterior lobe was exceptionally high. Throughout the present work high values for this region were occasionally met.

After a dose of 500 mg/Kg, there was no clear indication of a rise of histamine in the hypothalamus, thalamus or cerebral cortex of rabbit (1); although the values for the central grey matter and tegmental region showed an increase, they were lower than the increases observed after the infusion of a similar dose of histidine in the conscious animal. But in rabbit (2) the increases were similar to those seen in the conscious rabbit.

After a dose of 250 mg/Kg the rise of histamine in various areas of the brain was of the same order as that found after the infusion of the same dose in the conscious rabbit. All the values fell outside the fiducial limits of the untreated controls (P 0.05).

Table 21

Concentration of histamine in hypophysis, brain and blood after intravenous infusions of histidine in rabbits anaesthetized with pentobarbitone

Mean estimate ± S.E. expressed as nanograms per gram of fresh tissue or nanograms per ml. of whole blood (No. of rabbits).

	Dose of histidine mg/Kg 12hr	Anterior lobe of hypophysis	Hypothalamus	Medial thalamus	Central grey matter	Tegmental region	Reg. of pons and medulla	Caudate nucleus	Blood before infusion	Blood towards end of infusion
Untreated controls		650±36 (31)	660±27 (25)	270±18 (19)	280±15 (22)	170±12 (12)	140±15 (20)	150±12 (12)	4060±530 (11)	4000±530 (11)
Rabbit 1 Rabbit 2	500 500	690 790	760 1480	300 440	510 920	410 520	180 350		1900 3300	3300 3500
Histidine infused in conscious rabbits	500*	800±80 (11)	1480±130 (5)	560±70 (5)	960±70 (6)	440±30 (5)	260±30 (5)		3300±400 (10)	2900±500 (10)
Rabbit 3 Rabbit 4	250 250	1350 860	1080 1220		700 800	480 490	270 240	330 270	4300 3000	4000 2900
Histidine infused in conscious rabbits	250*	830±120 (5)	1360±120 (5)		800±70 (5)	460±20 (5)	280±20 (5)	290±20 (5)	3800±700 (5)	3700±800 (5)
Rabbit 5 Rabbit 6	62 62	880 770	730 900		350 440	260 270	180 170	190 200	3300 3300	2900 3300
Histidine infused in conscious rabbits	62*	880 (4)	1070 (4)		520 (4)	320 (4)	200 (4)	250 (4)	4000 (4)	4100 (4)

* Values taken from Table 18, page 100a.

After a dose of 62 mg/Kg an increase in the concentration of histamine was detected mainly in the midbrain, which was comparable with the increase found in the conscious rabbit. The rise of histamine in the hypothalamus, pons-medulla and caudate nucleus was less evident. In one experiment out of 4, the infusion of 62 mg/Kg in the conscious animal did not show a definite effect.

It can be concluded from these results that the anaesthetic did not interfere with histamine formation from exogenous histidine. The negative results in rabbit (1) are difficult to interpret.

5/5 GENERAL DISCUSSION

The rise of brain histamine seen after the infusion of histidine was probably due to local decarboxylation of the amino acid. Other factors, however, may have contributed to the result and are discussed below:

(a) Histamine in whole blood. Histidine may have been decarboxylated in formed elements of blood, particularly platelets (S. 1/5), thereby leading to a rise of histamine in whole blood. Residual blood in the cerebral vessels would then

increase the concentration of histamine extractable from brain. This assumption was tested in various ways and the following evidence was obtained:

(1) An increase in whole blood histamine after treatment with histidine was not detected (Table 18 and Fig. 6). Had there been an increase one might have expected to find a uniform concentration of the amine in various parts of the brain. But this was not so: the rise occurred unevenly and corresponded to the pattern of distribution for histamine.

(2) In 6 rabbits out of 9, infused with histidine (3 x 500 mg/Kg over 24 hr), the head was perfused with Ringer-Locke solution (S. 2/6). The concentration of histamine in brain was not diminished and the rise was comparable with that obtained after bleeding only (Table 18).

(3) Intravenous injection of glycogen (Appendix 3) caused a profound fall in the concentration of histamine in whole blood but not in the brain.

(b) Histamine in plasma. Decarboxylation of histidine by platelets and other tissues of the body may have raised the plasma histamine.

However, when histamine was infused intravenously the concentration in the hypothalamus and thalamus did not rise significantly (Table 19 and Fig. 7).

(c) Effect of i.v. infusion of hypertonic solution. Since the solution containing 30 mg histidine base/ml was hypertonic (S. 2/3), it might have reduced the water content of brain and so increased the concentration of histamine. However, when rabbits were infused with smaller doses of histidine in solutions that were isotonic (Table 7), the increase in histamine content was still evident (Table 18). Again, the i.v. infusion of hypertonic solution of histidine (30 mg/ml) in the cat (Appendix 1) did not cause a significant rise of histamine in the hypothalamus. Nonetheless, to exclude the possible effect of hypertonicity, sucrose solution in a concentration of 66 mg/ml (contributing 192 mosmoles/l and similar in osmolarity to that of histidine solution containing 30 mg/ml) was dissolved in normal physiological saline and infused. The results of these experiments (Table 22) were comparable with those of saline-infused controls.

Table 22

Effect of intravenous infusion of sucrose dissolved in saline* on the concentration of histamine in hypophysis and brain. Estimates expressed as nanograms/g.

	Saline infusion control					Sucrose dissolved in saline
	Mean	S.E.	S.D.	Limits (P0.05)	No.	(individual results)
Anterior lobe of hypophysis	580	50	170	470-700	II	750 720 1250 540
Tegmental region	180	6	14	160-190	6	190 140 160

* Concentration of sucrose: 66 mg/ml isotonic saline (0.9%)

Osmolarity of solution : 492 mosmoles/litre (300 contributed by saline + 192 by sucrose), which is similar to that of histidine solution containing 30 mg base/ml; (See S. 2/3).

(d) Decarboxylation of histidine during the extraction procedure. After repeated infusions of histidine, the concentration of the amino acid in brain and plasma was high (Appendix 2). Experiments (same Appendix) showed that histidine was not adsorbed on the resin-cellulose column (pH 8.0), nor was it converted to histamine in detectable amounts when exposed to various treatments involved in the method. Known quantities of histidine were also added to brain samples and extracted according to the usual procedure. The recovery of histamine from these extracts was similar to that of the control to which histidine was not added.

The results of all these experiments support the conclusion that most of the histamine extractable from brain of histidine-treated rabbits is histamine of tissue origin and not derived from residual blood or formed as an artifact from histidine.

Although the uptake of histidine by brain has been studied in other species both in vitro (Neame, 1961, 1962) and in vivo (Kamin and Handler 1951), similar studies have yet to be performed

in the rabbit. In the present work (Appendix 1) a high concentration of histidine in the rabbit brain was found after treatment with the amino acid. Since histamine disappears rapidly from the circulation (S. 1/9) and does not enter the brain in measurable quantities (S. 1/8, Table 19 and Fig. 7), it may be concluded that the rise of histamine concentration occurred because histidine entered the brain and was decarboxylated.

Histamine formation

After the i.v. infusion of histidine, the concentration of histamine increased in many parts of the rabbit brain (Table 18, Fig. 6). The percentage increase was highest in the midbrain, particularly in the central grey matter which was sensitive to small doses of histidine. The rise in the midbrain and hypothalamus after 250, 500 and 1500 mg/Kg of histidine was related to the dose. Increase in the concentration of histamine was also noted in all parts of the brain examined, especially after large doses. By contrast, the concentration did not rise detectably in the anterior lobe of the hypophysis or in blood.

Histamine formation in comparison with that of monoamines. The rise of brain histamine seen after the infusion of histidine indicates a rapid turnover of histidine to histamine which was greatest in the midbrain. This finding suggests that histamine may have an important role to play in brain function: "If the synthesis of histamine in the non-mast cell sites were to occur rapidly, it would be logical to propose that it might have a physiological function in the very tissues in which it is located" (Johnson, Beavan, Erjavec and Brodie, 1966). The present results confirm those of White (1959) who observed that the rate of histamine formation in hypothalamus of the cat was 10-15 times greater than in the cerebral cortex.

The rise of 5-HT in rabbit brain was maximal during the first two hours after the i.v. injection of 5-HTP (Costa and Rinaldi, 1958). Again, the rise of dopamine in rabbit brain after the i.v. injection of DOPA was maximal at 15-30 min (Bertler and Rosengren, 1959). Hence it would appear that histidine, in common with these amino acids, is rapidly decarboxylated in rabbit brain.

After treatment with 5-HTP, the concentration of 5-HT was highest in the midbrain (Costa and Rinaldi, 1958; Costa et al, 1960), hypothalamus and caudate nucleus (Udenfriend et al, 1957a); smaller rises were detected in the cerebral cortex and cerebellum (Udenfriend et al, 1957a; Costa and Rinaldi, 1958; Costa et al, 1960). After treatment with DOPA, the concentration of dopamine rose chiefly in the hypothalamus, caudate nucleus and midbrain (Bertler and Rosengren, 1959). Similarly, the rise of histamine was greatest in the hypothalamus and midbrain and least in the cerebral cortex and cerebellum (Table 18).

Subcellular localization of newly-formed histamine. Whether the newly-formed histamine is free or bound is not yet known. Were it 'free', one might expect it to disappear rapidly by catabolism. Since histamine formed from histidine (250-500 mg/Kg/2hr) persisted for at least 16 hr, it may have been stored in particles. This assumption could be tested by separating the particles and by estimating the catabolite 1,4-methyl-histamine.

Comparison of DC and INMT activities. According to Brown et al (1959) histamine methylating activity in the rabbit brain was more or less the same in the midbrain, cerebral cortex and cerebellum. This is in sharp contrast to the present findings (Table 18) which show that formation of histamine was greatest in the midbrain and least in the cerebellum. A similar lack of parallelism between formation and catabolism has been reported for the cat's brain (White, 1959).

Hypophysis. The finding that histidine did not cause a significant rise of histamine in the anterior lobe (Table 18) confirms earlier observations in the cat (Adam et al, 1964). Formation of histamine may have been too slow to be detectable under the conditions of the present experiments; alternatively, histamine may have diffused into the circulation.

The average histamine content of the posterior lobe (mean weight 5 mg) was about 2 ng (Table 13). After the infusion of large doses of histidine, the content rose to about 4 ng. When these values are expressed as percentages they look deceptively large. Since recovery of small

amounts of histamine (Table 8) from brain tissue by the present method was not quantitative, it is difficult to interpret the small rise in this part of the gland.

Concluding remarks. Histidine, DOPA and 5-HTP are decarboxylated in the cytoplasm (see Gaddum, 1956; Rosengren, 1960). During the shuttling between the cytoplasm and granules, histamine runs the risk of methylation (S. 1/7). The final concentration of histamine in cells may therefore represent the amine which has escaped catabolism (Green, 1962). "The likelihood of a highly organized system in the synthesis and intracellular transport of the amines is strengthened by observations suggesting that the cell may handle exogenous and endogenous amines differently" (Green, 1962); intact brain formed more MH from exogenous histamine than from endogenous histamine (White, 1960). The same cells that contain biogenic amines almost invariably contain enzymes that catabolize them. The activity of these enzymes is so high that the persistence of significant stores of amines would be most improb-

able unless the enzymes were either inactive in situ or not accessible to the amine.

The massive increase in brain histamine, which followed the infusion of histidine, was not accompanied by obvious pharmacological effects on behaviour; this might suggest that the amine was stored in granules. The slow disappearance of the newly-formed histamine (Fig. 8) supports this view.

Histamine uptake

After histamine infusion, the concentration did not rise significantly in the hypothalamus and thalamus (Table 19 and Fig. 7). These findings agree with those reported for the uptake of histamine by brain of other species (S. 1/8). In this respect histamine resembles other amines (S. 6/3).

Nevertheless, the concentration rose significantly in the cerebral cortex and cerebellum when histamine may have diffused from the pial vessels into the Virchow-Robin spaces and so entered the C.S.F. (Davson, 1956). In contrast, large doses of histidine did not produce a detectable rise of histamine in the anterior lobe and

had no apparent effect on behaviour. These findings indicate that if the plasma histamine had risen during the infusion of histidine, it was not sufficient to evoke pharmacological effects or to increase the concentration of histamine in the anterior lobe.

The marked rise of histamine in both lobes of the hypophysis supports the view that hypophysis is outside the blood brain barrier (Davson, 1956). The small increase in total blood histamine confirms earlier reports that histamine disappears rapidly from circulation (S. 1/9).

Duration of the rise in brain histamine.

The duration depended on the dose of histidine (Table 20, Fig. 8). After a dose of 62 mg/Kg, the histamine concentration in various parts of the brain had returned to the control value by 16 hr. After a dose of 500 mg/Kg, the concentration at 16 hr was still as high as at ½ hr. After a dose of 250 mg/Kg, the concentration began to fall at 16 hr and was within normal limits at 32 hr. It is probable (a) that the newly-formed histamine was 'bound' and therefore disappeared

slowly or (b) that the concentration of histidine in brain remained high for a long period of time during which decarboxylation continued, (c) or that both events occurred simultaneously.

In contrast, newly-formed 5-HT in rabbit brain returned to normal concentration 3 hr after the injection of 5-HTP (Costa and Rinaldi, 1958) the newly-formed dopamine about 1 hr after the injection of DOPA (Bertler and Rosengren, 1959). The rapid disappearance of these amines suggests that they were probably 'free', which could explain their marked effect on behaviour.

SECTION 6

EFFECTS OF VARIOUS AMINO ACIDS ON THE CONCENTRATION OF HISTAMINE IN RABBIT BRAIN, HYPOPHYSIS AND BLOOD AND THEIR INTERACTIONS WITH HISTIDINE.

Since 5-HT and dopamine are known to release each other from storage sites in rabbit brain (Bertler and Rosengren, 1959; Brodie et al, 1966), it was of interest to test whether treatment with precursors could alter the concentration of histamine.

It has been suggested that histidine DC of brain is 'non-specific' and resembles the enzyme from the guinea pig kidney (S. 1/6). In vitro, this enzyme has a strong affinity for 5-HTP and DOPA but a low affinity for histidine (Udenfriend et al, 1960; Weissbach et al, 1961; Lovenberg et al, 1962). If the same DC were responsible for the formation of histamine in rabbit brain, it would be expected that DOPA, 5-HTP, tryptophan and α-methyldopa (an inhibitor of the DC and a substrate for it) would interfere with histamine formation in vivo by competitive inhibition. Each of these amino acids was therefore infused simultaneously with histidine and histamine was

estimated in the following regions:

Anterior lobe

Hypothalamus

Central grey

Tegmental region

Pons-medulla

Caudate nucleus

Blood (before and after treatment)

SECTION 6/1

L-ALPHA-METHYLDOPA

The action of alpha-methyldopa (α-MD) on decarboxylase activity and on stores of monoamines in brain and other tissues has been frequently reviewed (for recent reviews, see Sourkes, 1965; Pletscher, Gey and Burkard, 1966).

Effect on DC enzyme. In vitro, α-MD inhibits L-aromatic amino acid DC of rabbit and guinea pig kidney (Mackay and Shepherd, 1960; Werle, 1961; Ganrot, Rosengren and Rosengren, 1961; Weissbach et al, 1961), but does not inhibit the histidine DC of mast cells (Weissbach et al, 1961) (Table 5). α-MD inhibits decarboxylation of 5-HTP both in vitro (Sourkes, 1954; Westermann,

Blazer and Knell, 1958) and *in vivo* (Dengler and Reichel, 1958), though the inhibitory effect *in vivo* is rather weak (Hess, Connamacher, Ozaki and Udenfriend, 1961; Brodie, Kuntzman, Hirsch and Costa, 1962; Drain, Horlington, Lazare and Poulter, 1962). *In vivo*, α-MD also inhibits decarboxylation of DOPA (Murphy and Sourkes, 1959).

Effect on concentration of monoamines in brain. In rabbit brain, α-MD lowers the concentration of noradrenaline, dopamine (Carlsson and Lindqvist, 1962) and 5-HT (Carlsson and Lindqvist, 1962; Roos and Werdinius, 1963). The concentration of noradrenaline remains low for many days and long after any α-MD is detectable in brain (Carlsson and Lindqvist, 1962); though the concentrations of 5-HT and dopamine recover rapidly (Smith, 1960; Leroy, 1961).

Mechanism of monoamine depletion. It has been suggested (Brodie et al, 1962) that DC inhibition per se does not cause depletion of noradrenaline in brain. This view is supported by the finding that hydroxyphenylalkylhydrazines (NSD compounds) which are potent inhibitors of DC activity, both *in vitro* and *in vivo*, do not

lower the concentration of noradrenaline in guinea pig brain (Levine and Sjoerdsma, 1964). Moreover, when a-MAO inhibitor was given at the time of initial depletion of noradrenaline by α-MD, the concentration began to rise, indicating the ability of brain to synthesise the amine during this period (Hess et al, 1961). It has been assumed however that catecholamines in brain are displaced by α-methyldopamine and α-methylnoradrenaline, since α-MD is decarboxylated *in vitro* (Weissbach et al, 1961; Lovenberg et al, 1962) and *in vivo* (Carlsson and Lindqvist, 1962). This hypothesis is supported by the finding that α-methylated amines are not attacked by MAO which may explain why they compete effectively for the stores (Carlsson and Lindqvist, 1962). This conclusion is further substantiated by the finding that NSD compounds prevent the action of α-MD on noradrenaline, presumably by inhibiting the decarboxylation of α-MD (Levine and Sjoerdsma 1964).

The mechanism of 5-HT depletion is more difficult to explain. It was suggested that α-MD acts by inhibiting the synthesis of 5-HT

in brain, since it caused simultaneous decrease of 5-HT and 5-HIAA (Roos and Werdinius, 1963): "In the case of displacement or release of 5-HT an increase rather than decrease in 5-HIAA should occur. The fact that 5-HIAA appeared to decrease more slowly than 5-HT might possibly indicate some displacement or release in addition to inhibition of synthesis". The inhibition of hydroxylation of tryptophan may be another contributory factor (Burkard, Gey and Pletscher, 1964).

Effect on exogenous aromatic amino acids. In rat brain α-MD reduces the rise of monoamines seen after the administration of the corresponding precursors, namely, 5-HTP (Kunz, 1964) and DOPA (Sourkes and Murphy, 1960; Murphy and Sourkes, 1961). Schayer and Sestokas (1965) found that pre-treatment of guinea-pigs with α-MD reduced the amount of labelled urinary histamine formed from ^{14}C-histidine.

It was desirable therefore to study the effect of α-MD on the concentration of histamine in brain and its interaction with the effect of histidine.

6/1/1 Materials and method

Single infusion of L-3-(3,4-dihydroxyphenyl)-2-methylalanine. 1½ H_2O (α-methyldopa) (α-MD) (Aldomet) (Merck Sharp and Dohme, powdered for research purposes). Preparation of α-MD solution for i.v. infusion is described in S. 2/3 and shown in Table 7. Rabbits received 200 mg/Kg/2hr and were anaesthetized 30 min after the end of infusion. For simultaneous infusion of α-MD (200 mg/Kg) and histidine (250 mg/Kg), both amino acids were neutralized and dissolved in water (osmolarity 320 mosmoles/l).

Repeated infusions of α-MD (4 x 200 mg/Kg over 36 hr). Each rabbit received 4 infusions of α-MD suspension (20 mg/ml) over a period of 36 hr. A dose of α-MD (200 mg/Kg) was given every 12 hr and the animal anaesthetized 30 min after the end of the last infusion. The amino acid was infused at the rate of 20 mg/min.

In another set of experiments rabbits received ~~an~~ infusions of α-MD suspension (200 mg/Kg) every 12 hr over a 36 hr period the 4th dose was infused simultaneously with histidine (250 mg/Kg) over 2 hr.

6/1/2 Results

Rabbits showed signs of sedation about 30 min after the second infusion: slight constriction of pupils and blepharospasm. Recovery was rapid. The rectal temperature was unchanged.

Effect on histamine concentration. A single infusion of α-MD produced no detectable change in the concentration of histamine extractable from blood, hypophysis or brain. After repeated infusions, the concentration rose in the midbrain only; values for the central grey and tegmental region fell outside the limits of the control (Table 23 and Fig. 9).

Single or repeated infusions of α-MD did not seem to affect the formation of histamine from histidine. The concentration of histamine in brain was still as high as that obtained after the infusion of histidine alone. (Table 24).

SECTION 6/2

L-DIHYDROXYPHENYLALANINE

The i.v. injection of DL-DOPA (100 mg/Kg) in rabbits raised the concentration of dopamine mainly in the hypothalamus and caudate nucleus, and produced central stimulation and increased motor

TABLE 23

Effect of intravenous infusion of L-α-METHYLDOPA on the concentration of histamine in hypophysis, brain and blood of rabbit.

	Dose	Estimates expressed as ng/g of tissue or ng/ml of blood							
		Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Tegmental region	Reg. pons medulla	Caudate nucleus	Whole blood	
								before treatment	before sacrifice
Rabbit 1	200mg/kg/2hr; rabbits anaesthetized ½hr after infusion.	850	790	370	250	180	160	4300	3300
Rabbit 2	200mg/kg/½hr; 4 doses given over 36hr; rabbits anaesthetized ½hr after last dose.	1290	670	470	340	200	210	2500	3200
Rabbit 3	As rabbit 2.	590	730	380	270	270	150	3300	3800
Rabbits 1-3 Mean		910	730	410	290	220	170	3400	3400
Control Mean ± S.E. (No.)	-	650 ± 36 (31)	660 ± 27 (25)	280 ± 15 (22)	170 ± 12 (12)	140 ± 15 (20)	150 ± 12 (12)	4060 ± 530 (11)	4000 ± 530 (11)

Table 26

Effect of simultaneous infusion of L-α-METHYLDOPA and L-HISTIDINE on the concentration of histamine in hypophysis, brain and blood of rabbit. Rabbits anaesthetized ½ hr after the end of last infusion. (No. of rabbits).

	Dose of histidine, mg/Kg/2 hr	Dose of α-methyldopa, mg/Kg	Estimates expressed as ng/g of tissue or ng/ml of blood							
			Anterior lobe of hypophysis	hypothalamus	Central grey matter	Tegmental region	Reg. pons and medulla	Caudate nucleus	Whole Blood	
									before treatment	before sacrifice
Rabbit 1	250	200	1020	1000	560	350	210	240	3000	3000
Rabbit 2	250	4x200*	760	1260	730	510	250	310	2200	2500
Rabbit 3	250	4x200*	730	1340	560	400	290	290	4300	4300
Rabbits 1-3 Mean			840	1200	620	420	250	280	3200	3300
α-methyldopa alone[1] Mean		200-4x200	910 (3)	730 (3)	410 (3)	290 (3)	220 (3)	170 (3)	3400 (3)	3400 (3)
Histidine alone[2] Mean±S.E.	250		830±120 (5)	1360±120 (5)	800±70 (5)	460±20 (5)	280±20 (5)	290±20 (5)	3800±700 (5)	3700±800 (5)
Untreated control Mean±S.E.			650±36 (31)	660±27 (25)	280±15 (22)	170±12 (12)	140±15 (20)	150±12 (12)	4060±530 (11)	4000±530 (11)

* 3 doses given every 12 hr; the last dose infused simultaneously with histidine

[1] From Table 25, page 127a

[2] From Table 18, page 100a

activity (Carlsson, Lindqvist, Magnusson and Waldeck, 1958; Bertler and Rosengren, 1959). Pharmacological effects appeared to be closely related to the accumulation of dopamine (Bertler and Rosengren, 1959). These authors also found that repeated injections of DOPA (600 mg/Kg total) during a 2 hr period lowered the concentration of 5-HT in brain to 50-60% of the control.

It was of interest to study the effect of DOPA on the concentration of histamine in brain and on the formation of histamine.

6/2/1 Materials and method

Preparation of L-β-(3,4-dihydroxyphenylalanine) L-DOPA) (Koch-Light Lab. Ltd., Batch No. 31784) solution for i.v. infusion has been described (S. 2/3, Table 7). Rabbits received 60-100 mg/Kg/2 hr and were observed for gross changes in behaviour. For simultaneous infusion of L-DOPA (60 mg/Kg) and histidine (250 mg/Kg), the two amino acids were dissolved in the same vehicle (osmolarity 290 mosmoles/l).

6/2/2 Results

Four rabbits were used; one died at the height of hyperpyrexia.

After about 1 hr when 30-50 mg/Kg had been infused, a slight dilatation of the pupils was noted. Gradually the animal became very sensitive to touch and sound stimuli; the pupils became widely dilated and the respiration very rapid. Towards the end of infusion, increased motor activity, salivation and constriction of ear vessels were observed. By this time rectal temperature had risen by 2-3°C. Thirty minutes after the end of infusion the animal was still excited.

When L-DOPA was infused simultaneously with histidine, pharmacological signs of central stimulation produced by L-DOPA alone were much less pronounced and rectal temperature remained unchanged. This observation may be explained in several ways: (a) histidine may have competed with DOPA for the entry into the brain, (b) histidine may have competed with DOPA for the DC enzyme in brain and so lowered the amount of catecholamines formed or (c) histamine may have counteracted the pharmacological effects of catecholamines. These speculations can only be tested by further experiments.

Effect on histamine concentration. Only the regions of the central grey and tegmentum showed

Table 25

Effect of intravenous infusion of L-DOPA on the concentration of histamine in hypophysis, brain and blood of rabbit. Animals anaesthetized ½ hr after the end of infusion.

	Dose	Estimates expressed as ng/g of tissue or ng/ml of blood							
		Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Tegmental region	Reg. pons and medulla	Caudate nucleus	Whole blood	
								Before infusion	Towards end of infusion
Rabbit 1	100 mg/kg/2 hr	880	710	440	230	170	270	3800	3800
Rabbit 2	60 mg/kg/ 2 hr	1120	720	470	260	160	190	4100	4100
Control Mean ± S.E. (No.)	-	650 ± 36 (31)	660 ± 27 (25)	280 ± 15 (22)	170 ± 12 (12)	140 ± 15 (20)	150 ± 12 (12)	4060 ± 530 (11)	4000 ± 530 (11)

TABLE 26

Effect of simultaneous infusion of L-DOPA (60 mg/kg/2hr) and L-HISTIDINE (250 mg/kg/2hr) on the concentration of histamine in hypophysis, brain and blood of rabbit. Animal anaesthetized ½hr after the end of infusion. (No. of rabbits)

	Estimates expressed as ng/g of tissue or ng/ml of blood.							
	Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Tegmental region	Reg. pons medulla	Caudate nucleus	Whole blood	
							Before Infusion	Towards end of infusion.
Rabbit 1 (dopa + histidine)	730	1140	540	360	330	200	4300	4300
Dopa alone [1] (60-100mg/kg/2hr) Mean	1000 (2)	720 (2)	460 (2)	250 (2)	170 (2)	230 (2)	4000 (2)	4000 (2)
Histidine alone[2] (250mg/kg/2hr) Mean ± S.E.	830 ±120 (5)	1360 ± 120 (5)	800 ± 70 (5)	460 ± 20 (5)	280 ± 20 (5)	290 ± 20 (5)	3800 ± 700 (5)	3700 ± 800 (5)
Untreated controls Mean ± S.E.	650 ± 36 (31)	660 ± 27 (25)	280 ± 15 (22)	170 ± 12 (12)	140 ± 15 (20)	150 ± 12 (12)	4060 ± 530 (11)	4000 ± 530 (11)

[1] From Table 25

[2] From Table 18

a slight rise (Table 25, Fig. 9). L-DOPA did not interfere with the formation of histamine in brain when infused together with histidine; the rise was of the same order as that seen after histidine alone, (Table 26).

SECTION 6/3

DL-5-HYDROXYTRYPTOPHAN

The i.v. injection of 5-HTP (22-100 mg/Kg) in rabbits raised the concentration of 5-HT in brain (Udenfriend et al, 1957; Costa and Rinaldi, 1958; Bertler and Rosengren, 1959; Costa et al, 1960). It has been shown that labelled 5-HTP readily enters the rabbit brain (Udenfriend et al, 1957; Sankar, Phipps, Gold and Sankar, 1962). Since 5-HT is rapidly catabolized and does not enter the brain in measurable quantities, it was concluded that 5-HT is normally synthesised in the brain (Udenfriend et al, 1957).

It has been reported that 5-HT lowers the concentration of histamine in rabbit blood in vivo (Burkhalter et al, 1960) and in tissues of other species (Feldberg and Smith, 1953). 5-HT is also known to release noradrenaline from rabbit brain

(Brodie et al, 1966). It is therefore possible that amines are able to release each other from tissues.

It is also known that 5-HT inhibits methylation of histamine *in vitro* (Brown et al, 1959; Gustafsson and Forshell, 1963) and *in vivo* (Snyder and Axelrod, 1964). It was conceivable therefore that accumulation of 5-HT in brain could alter the concentration of histamine by release from stores or by inhibition of its catabolism.

Monnier and Tissot (1958) and Monnier (1960) observed slight sedation in the rabbit after the i.v. injection of 10-20 mg/Kg 5-HTP. Signs of sympathetic stimulation were seen after 40-200 mg/Kg (Udenfriend et al, 1957; Bogdanski, Pletscher, Brodie and Udenfriend, 1956; Bogdanski, Weissbach and Udenfriend, 1958; Costa and Rinaldi, 1958; Brodie et al, 1966). Large doses have a strong pyretogenic effect in the rabbit, raising the rectal temperature to 43-44°C (Horita and Gogerty, 1958).

6/3/1 Materials and method

Preparation of DL-5-hydroxytryptophan (5-HTP) (Koch-Light Lab. Ltd., Batch No. 29397) solution for i.v. infusion has been referred to (S. 2/3, Table 7).

Rabbits received 63-75 mg/Kg/2 hr and were observed for changes in behaviour. For simultaneous infusion of 5-HTP and histidine, both amino acids were dissolved in water (osmolarity 290 mosmoles/l).

6/3/2 Results

Seven rabbits were used; one died of hyperpyrexia.

During the early part of infusion the animal was quiet. The first sign of excitation (dilatation of pupils) was observed after the infusion of about 40 mg/Kg or more. Gradually pupils became more widely dilated and rabbit became restless and easily excitable; other pharmacological signs included increased respiratory rate, tremors, twitching and uncoordinated movements of the limbs. Two rabbits had fits and when the infusion was stopped they recovered rapidly. Rectal temperature taken after the end of infusion rose by 1.5-4°C.

Effect on the concentration of histamine. The concentration rose significantly only in the central grey matter and tegmental region (Table 27, Fig. 9). Infusion of 5-HTP (75 mg/Kg) together with histidine (250 mg/Kg) did not interfere with the formation of histamine in brain (Table 28).

Table 27

Effect of DL-5-HTP infusions on the concentration of histamine in hypophysis, brain and blood of rabbit

	Total dose in mg/Kg/ 2 hr.	Estimates expressed as ng/g fresh tissue or ng/ml blood							
		Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Reg. Tegmentum	Caudate nucleus	Reg. pons and medulla	Whole Blood	
								before infusion	Towards end of infusion
Rabbit 1	75	710	720	470	240	190	160	2700	2700
Rabbit 2	75	450	1360	760	410	310	300	4800	4800
Rabbit 3	63	840	800	360	200	140	160	4300	3500
Rabbit 4	64	1290	580	290	250	120	140	3300	2700
Rabbit 5	65	910	530	450	240	120	100	3300	2200
Rabbits 1-5 Mean±S.E.		840±140	800±150	470±80*	270±40†	180±40	170±30	3700 ± 380	3200 ± 460
Controls Mean±S.E. (no. of rabbits)		650±36 (31)	660±27 (25)	280±15 (22)	170±12 (12)	150±12 (12)	140±15 (20)	4060±530 (11)	4000±530 (11)

* Significantly different from mean of control $P < 0.001$

† Significantly different from mean of control $P < 0.01$

Table 28

Effect of simultaneous infusion of DL-5-HTP (75 mg/Kg/2 hr) and L-histidine (250 mg/Kg/2 hr) on the concentration of histamine in hypophysis, brain and blood of rabbit. Animal anaesthetized ½ hr after the end of infusion. (No. of rabbits).

	Estimates expressed as ng/g of tissue or ng/ml of blood							
	Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Tegmental region	Reg. pons and medulla	Caudate nucleus	Whole Blood	
							before infusion	towards end of infusion
Rabbit 1 (5-HTP+histidine)	730	1250	810	520	300	370	5400	4400
5-HTP alone[1] (63-75mg/Kg/2 hr) Mean ± S.E.	840±140 (5)	800±150 (5)	470±80 (5)	270±40 (5)	170±30 (5)	180±40 (5)	3700±380 (5)	3200±460 (5)
Histidine alone[2] (250 mg/Kg/2 hr) Mean ± S.E.	830±120 (5)	1360±120 (5)	800±70 (5)	460±20 (5)	280±20 (5)	290±20 (5)	3800±700 (5)	3700±800 (5)
Untreated controls Mean ± S.E.	650±36 (31)	660±27 (25)	280±15 (22)	170±12 (12)	140±15 (20)	150±12 (12)	4060±530 (11)	4000±530 (11)

[1] From Table 27, page 130a

[2] From Table 18, page 100a

SECTION 6/4

L-TRYPTOPHAN

Tryptamine is found in brain in low concentrations (Hess and Doepfner, 1961). Hess, Redfield and Udenfriend (1959) reported that in rabbits pre-treated with iproniazid, L-tryptophan produced central effects and a marked increase in the concentration of tryptamine in brain.

Tryptophan is decarboxylated to tryptamine both *in vitro* (Lovenberg et al, 1962) and *in vivo* (Hess et al, 1959). Another route of tryptophan metabolism is its hydroxylation to 5-HTP (see Hagen and Cohen, 1966). Labelled 5-HT has been found in animal tissues after the administration of radioactive tryptophan (Udenfriend and Weissbach, 1958).

Tryptamine is known to lower the concentration of histamine in tissues of dog, cat and rat (Feldberg and Smith, 1953). The object was therefore to test the effect of tryptophan on the concentration of histamine in brain.

6/4/1 Materials and method

Solution for i.v. infusion was prepared as described in S. 2/3 (Table 7).

Table 29

Effect of intravenous infusion of L-Tryptophan (200 mg/Kg/2 hr.) on the concentration of histamine in hypophysis, brain and blood. Rabbits anaesthetized ½ hr. after the infusion.

	Estimates expressed as ng/g of tissue or ng/ml of blood							
	Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Tegmental region	Reg. pons of medulla	Caudate nucleus	Whole blood/le blood	
							Before infusion	Towards end of infusion
Rabbit 1	630	770	360	230	230	130	3300	3300
Rabbit 2	520	610	300	200	140	140	3300	2300
Control mean ± S.E.	650 ± 36	660 ± 27	280 ± 15	170 ± 12	140 ± 15	150 ± 12	4060 ± 530	4000 ± 530
(No.)	(31)	(25)	(22)	(12)	(20)	(12)	(11)	(11)

6/4/2 Results

The infusion of L-tryptophan (200 mg/Kg) did not produce any gross pharmacological changes in behaviour, nor did it alter the concentration of histamine extractable from blood, hypophysis or brain (Table 29, Fig. 9).

SECTION 6/5

GENERAL DISCUSSION

The amino acids, α-MD, DOPA, 5-HTP and tryptophan did not alter the concentration of histamine in the hypophysis. 5-HTP lowered the concentration in blood, which confirms the observation by Burkhalter et al (1960). In the central grey and tegmental regions, the concentration increased by about 50-70% after treatment with α-MD, DOPA or 5-HTP (Fig. 9). Simultaneous infusions of α-MD, DOPA or 5-HTP with histidine did not interfere with the formation of histamine in brain. (Tables 24, 26 and 28).

α-Methyldopa. The rise of histamine in midbrain (Table 23) is in sharp contrast to the fall in the concentration of monoamines caused by α-MD (S. 6/1). The same doses of α-MD used in the present work caused almost complete disappearance

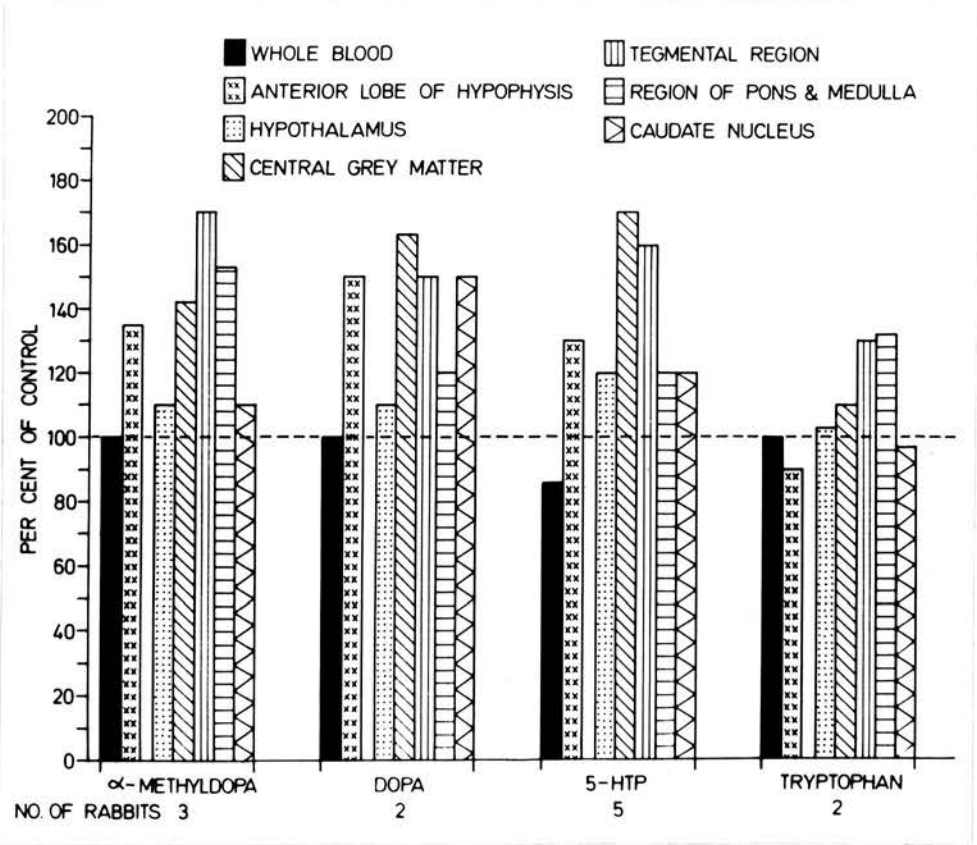


Fig. 9. Effect of i.v. infusions of various amino acids on the concentration of histamine in blood, anterior lobe of hypophysis and different regions of the brain, expressed as a percentage of the control. Rabbits were anaesthetized ½ hr after the end of infusion and then sacrificed. Doses of amino acids:
α-Methyldopa: 200 mg/Kg/2 hr (single experiment) or 4 infusions of 200 mg/Kg/30 min. at 12 hourly intervals (2 experiments).
DOPA: 60 and 100 mg/Kg/2 hr
5-HTP: 63-75 mg/Kg/2 hr
Tryptophan: 200 mg/Kg/2 hr.

of noradrenaline from the rabbit brain (Carlsson and Lindqvist, 1962). The present results suggest that products of decarboxylation of α-MD do not displace histamine from its storage sites, which might indicate that the binding mechanism or the storage sites of histamine are different from those of monoamines.

The finding that α-MD did not interfere with the formation of histamine from exogenous histidine might suggest that α-MD did not inhibit the DC for histidine. By comparison, pre-treatment with α-MD reduces the formation of monoamines from their respective precursors (S. 6/1).

The rise of histamine in midbrain could be explained in terms of inhibition of histamine catabolism by metabolic products of α-MD or the released monoamines. The 'free' 5-HT released by α-MD might have inhibited the methylation of histamine (S. 6/3). Until more is known about the chanacteristics of histidine DC in the rabbit brain, one can only speculate about the present results.

DOPA and 5-HTP. The rise of histamine in the midbrain seen after the infusion of these amino acids

could be due to inhibition of histamine catabolism by the monoamines formed or their catabolites.

When either of these amino acids was infused with histidine, it did not modify the formation of histamine in brain. It is difficult to explain this finding since it is not known to what extent, if any, histidine interfered with the entry of DOPA or 5-HTP into the brain. If we can assume that the entry of DOPA or 5-HTP was not retarded, then it seems probable that they did not inhibit the decarboxylation of histidine by competing for the DC enzyme. It has been reported however that histidine, in comparison with DOPA and 5-HTP, is a very poor substrate for aromatic L-amino acid DC (S. 6). The present results raise the question whether or not histidine is decarboxylated by the so-called 'non-specific enzyme' in brain or by a separate enzyme specific for histidine. Because we were not able to estimate histidine and monoamines in the brain after the above treatments, we cannot draw any definite conclusions from these results.

SECTION 7

EFFECT OF DRUGS ON THE CONCENTRATION OF HISTAMINE IN RABBIT BLOOD, HYPOPHYSIS AND BRAIN

The aim was to test the effect of certain psychotropic drugs on the concentration of histamine in blood, hypophysis and various parts of the brain. The drugs chosen are known to affect the concentration of biogenic amines in brain. These studies also included the interaction of drugs with the effect of histidine.

SECTION 7/1

CHLORPROMAZINE

A large number of phenothiazine derivatives show varying degrees of tranquilizing, antiemetic and antihistaminic properties (see Parkes, 1961). Chlorpromazine (CPZ), however, has little antihistaminic activity and is better known for its central actions (Courvoisier, Fournel, Ducrot, Kolsky and Koetschet, 1953).

Effect on metabolism of monoamines. CPZ has no apparent effect on the concentration of monoamines in rabbit brain (Brodie, Shore and Pletscher, 1956; Costa and Rinaldi, 1958; Anden, Roos and

Werdinius, 1964), but increases 5-HT content of brain in mice and rats (Bartlet, 1960; Costa, Garattini and Valzelli, 1960). CPZ is also reported to influence the changes in the concentration of monoamines induced by other drugs (Costa and Rinaldi, 1958; Gey and Pletscher, 1961; Sankar et al, 1962). Furthermore, it increases the concentration of dopamine catabolites (dihydroxyphenylacetic acid and homovanillic acid) in rabbit brain, but does not affect the 5-HIAA content (Anden et al, 1964).

Effect on metabolism of histamine. CPZ inhibits INMT in vitro (Brown et al, 1959; Gustafsson and Forshell, 1963). In vivo, large doses of CPZ reduced the formation of labelled MH in cat's brain when ^{14}C-histamine was perfused through the cerebral ventricles (White, 1961a). CPZ has been reported to increase the concentration of histamine in brain of cat (Adam and Hye, 1966; White, 1966) and rat (Ungar and Witten 1963; Green and Erickson 1964; Adam, unpublished results).

Metabolism of CPZ. Has been reviewed by Emmerson and Miya (1963). CPZ was detected in rat brain long after the injection of the drug (Weschsler

and Forrest, 1959) and was concentrated in the hypothalamus (Wase, Christensen and Polley, 1956). It is therefore possible that large doses of CPZ would raise the concentration of the drug in the rabbit brain sufficiently to inhibit methylation of histamine thus leading to its accumulation. It was also desirable to test whether pre-treatment with CPZ would influence the formation of histamine from exogenous histidine.

7/1/1 Materials and method

10-(3-dimethylaminopropyl)-2-Chlorphenothiazine-HCl (Chlorpromazine HCl) (CPZ) (Largactil) (May and Baker Ltd.) was dissolved in sterile physiological saline to make 5 mg base/ml. The drug was freshly prepared for injection. In the first set of experiments each rabbit received 3 x 10 mg/Kg over 24 hr., the last dose was given 2 hr before sacrificing the animal. CPZ solution was injected slowly into the marginal ear vein. In the second set, rabbits were given the same doses of CPZ; the last dose, injected at 22 hr, was immediately followed by the infusion of histidine (250 mg/Kg/2 hr) and animals were sacrificed 30 min after the end of infusion.

7/1/2 Results

Signs of sedation were observed soon after the first dose of CPZ: constriction of the pupils, blepharospasm, relaxation of the nictitating membranes and general flaccidity of the muscles. About 1 hr after the injection, respiration became slow and deep and the animals lay prostrate in the cage; rectal temperature dropped by 2-3°C. At the time of killing the rabbits were deeply sedated but could be easily roused. The dose of CPZ employed was close to the LD_{50} (15 mg/Kg i.v., 'Handbook of Toxicology', 1959). When the animals were anaesthetized with pentobarbitone, they required less anaesthetic and less time in comparison with untreated rabbits.

Sedation and hypothermia were not changed when rabbits were infused with histidine.

Effect on the concentration of histamine. The mean concentration rose in the hypothalamus and central grey, but not in the pons-medulla or hypophysis (Table 30, Fig. 10). In the hypothalamus, the concentration increased significantly ($P < 0.05$) to 130% of the control; in the midbrain to 170% ($P < 0.001$). CPZ did not influence the formation of

TABLE 30

Effect of intravenous injections of Chlorpromazine (3 x 10 mg/kg over 24 hr) on the concentration of histamine in hypophysis, brain and blood of rabbit. (No. of rabbits).

	Estimates expressed as ng/g fresh tissue or ng/ml blood					
	Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Reg. pons and medulla	Whole Blood	
					Before treatment	Before sacrifice
Rabbit 1	1160	790	410	220	2400	2400
Rabbit 2	760	1070	690	140		
Rabbit 3	700	890	460	190		
Rabbit 4	530	970	510	140		
Rabbit 5	950	490	280	110		
Rabbits 1-5 Mean ± S.E.	820 ±110	840 ± 100 *	470 ± 70 †	160± 20		
Controls Mean ± S.E.	650 ± 36 (31)	660 ± 27 (25)	280 ± 15 (22)	140 ±15 (20)		

* Significantly different from Mean of Control $P < 0.05$

† Significantly different from Mean of Control $P < 0.001$

TABLE 31

Effect of histidine on the concentration of histamine in hypophysis and brain of rabbits pre-treated with chlorpromazine:

Chlorpromazine (3 x 10 mg/kg i.v. over 22 hr) + Histidine (250 mg/kg/2 hr) infused soon after the last injection of chlorpromazine. Rabbits sacrificed about ½ hr. after the infusion. (No. of animals)

	Estimates expressed as ng/g of fresh tissue.			
	Anterior lobe of hypophysis	Hypothalamus	Gentral grey matter.	Reg. pons and medulla
Rabbit 1	1950	1270	710	280
Rabbit 2	540	1240	600	270
Rabbit 3	610	1150	660	350
Rabbits 1-3 mean	1030	1220	660	300
Chlorpromazine alone[1] (3 x 10 mg/kg i.v. over 24 hr) Mean ± S.E.	820 ± 110 (5)	840 ± 100 (5)	470 ± 70 (5)	160 ± 20 (5)
Histidine alone[2] (250 mg/kg/2hr) at ½ hr Mean ± S.E.	830 ± 120 (5)	1360 ± 120 (5)	800 ± 74 (5)	280 ± 20 (5)
Untreated Controls Mean ± S.E.	650 ± 36 (31)	660 ± 27 (25)	280 ± 15 (22)	140 ± 15 (20)

[1] From Table 30

[2] From Table 18

histamine from histidine (Table 31).

7/1/3 Discussion

CPZ increased the concentration of histamine in the midbrain and hypothalamus and may have acted by interfering with its catabolism. Since CPZ inhibits methylation of histamine *in vitro* (Brown et al, 1959; Gustafsson and Forshell, 1963) and *in vivo* (White, 1961; Snyder and Axelrod, 1964), "it may have acted in this way to increase the brain histamine" (Adam and Hye, 1966). The present results in the rabbit agree with those reported for the cat (Adam and Hye, 1966; White, 1966) and rat (Ungar and Witten, 1963; Green and Erickson, 1964; Adam, unpublished results). By contrast, treatment with CPZ has no apparent effect on the concentration of monoamines in rabbit brain (S. 7/1).

Apart from inhibiting methylation, CPZ may have acted on the storage mechanism of histamine, as has been suggested for other amines (see Pletscher, 1963). Moreover, CPZ is known to inhibit several enzyme systems (Abood, 1955; Magee, Berry and Rossiter, 1956; Century and Horwitt, 1956; Brown et al, 1959; Decsi, 1961). Hence "the effect of the phenothiazines in raising the brain histamine is not

necessarily related to the central actions of the drugs, since the large doses employed could have acted in other ways", (Adam and Hye, 1966).

The hypophysis also methylates histamine (Axelrod et al, 1961), but the concentration in the anterior lobe did not rise after treatment with CPZ (Table 30). Since this region is devoid of a 'blood-brain barrier' (Davson, 1956), histamine may have diffused into the blood. Alternatively, the turnover rate for histamine in the hypophysis may be slower than that in the brain.

The finding that CPZ did not influence the formation of histamine from exogenous histidine may indicate that the newly-formed amine was mainly 'bound'. If it were 'free' and accessible to the methylating enzyme, then CPZ would have acted to protect it from methylation, thus leading to an increase in the concentration of histamine greater than that observed after histidine alone. In contrast, when rabbits were pretreated with CPZ, the administration of 5-HTP caused increases of 5-HT concentration in various areas of the brain which were greater in magnitude than those found in animals treated with 5-HTP alone (Costa and Rinaldi, 1968).

SECTION 7/2

IPRONIAZID

Effect on metabolism of histamine. MH is oxidatively deaminated by MAO to MeImAA (S. 1/7, Fig. 5). This route can also be influenced by certain psychotropic drugs.

The subcutaneous injections of iproniazid (IPN) in the cat completely inhibited the oxidation of MH in the brain after perfusion of ^{14}C-histamine through the cerebral ventricles (White, 1960). Repeated injections of IPN in the cat raised the concentration of histamine in brain but not in the hypophysis (Adam and Hye, 1966). These results differ from those reported by White (1966) who found that IPN raised the concentration of MH in brain but not of histamine.

Effect on monoamines. IPN is known to inhibit MAO enzyme of rabbit brain both *in vitro* and *in vivo*. After a single dose of 100 mg/Kg, the MAO activity was completely inhibited within ½ hr and remained so for 4 days (Spector, Shore and Brodie, 1960; Spector, 1963). The same dose raised the concentration of 5-HT in the rabbit brain; the concentration of noradrenaline rose more slowly and

to a lesser extent (Bogdanski et al, 1956; Spector et al, 1960). Daily subcutaneous injections of IPN (25 mg/Kg) increased the concentration of 5-HT in rabbit brain which was 2-3 times normal in 2 days; maximal concentration of noradrenaline was reached after 4 days. The rise of noradrenaline was accompanied by sympathomimetic signs (Brodie et al, 1959). Repeated injections of IPN raised the concentration of 5-HT and noradrenaline in hippocampus, midbrain and hindbrain of the rabbit (Himwich, Costa, Pscheidt and Van Meter, 1959; Costa et al, 1960).

Metabolism of IPN. When 5 mg/Kg labelled IPN was given by intraperitoneal injection to rabbits (Koechlin and Iliev, 1959), 60% of radioactivity was found in the expired CO_2; half-life of radioactivity was 6 hr.

The object was to test whether treatment with IPN would alter the concentration of histamine in brain or whether it would influence the formation of histamine from exogenous histidine.

7/2/1 Materials and method

I-isonicotinyl-2-isopropylhydrazine-phosphate (Iproniazid) (IPN) (Marsilid) (Roche Products Ltd.)

was dissolved in water to make 50 mg base/ml.

Eleven rabbits were used. Rabbits 6 and 7 (Table 32) received a single i.v. injection of IPN (100 mg/Kg) and were killed at 22 hr. Rabbits 1-5 received a total of 125 or 250 mg/Kg as a daily subcutaneous injection of 25 or 50 mg/Kg on 5 successive days; they were sacrificed 2 hr after the last injection.

Two more rabbits (Table 33) received 5 daily injections of 25 mg/Kg/day; immediately after the last dose they were infused with histidine (250 mg/Kg/2 hr) and killed 30 min after infusion.

7/2/2 Results

After repeated injections of IPN rabbits became weak and on the 5th day they were excitable and had lost some weight; mucous membranes were slightly cyanosed; there was no change in rectal temperature. One rabbit died on the 5th day after receiving a total dose of 200 mg/Kg; LD_{50} for IPN in rabbit is 117 mg/Kg i.v. (Handbook of Toxicology, 1959). On bleeding, the colour of blood was found to be deep red to dark.

Histidine infusion on the 5th day did not change the condition of the animals.

Effect on histamine concentration. After repeated injections of IPN (Table 32, Fig. 10), the concentration rose only in the central grey matter; the 40% increase was significant ($P < 0.01$).

IPN did not interfere with the formation of histamine from histidine (Table 33).

7/2/3 Discussion

Treatment with IPN increased the concentration of histamine in the midbrain but not in the hypothalamus, hindbrain or hypophysis.

IPN does not inhibit methylation of histamine (Schayer and Karjala, 1956; Lindahl, 1960), but does inhibit the oxidation of MH (White, 1960) which could lead to the accumulation of this catabolite in brain. Treatment with IPN also increases the concentration of 5-HT in the rabbit brain (S. 7/2). Since it is known that both MH (Brown et al, 1959; Lindahl, 1960) and 5-HT (Brown et al, 1959; Snyder and Axelrod, 1964) inhibit methylation of histamine, it can be argued tentatively that under the conditions of the present experiments, IPN acted indirectly through the accumulation of MH and 5-HT to raise the brain histamine (Adam and Hye, 1966).

Table 32

Effect of iproniazid on the concentration of histamine in hypophysis, brain and blood of rabbit (no. of rabbits)

	Total dose in mg/Kg	Period of treatment, days	Estimates expressed as ng/g of fresh tissue or ng/ml of blood					
			Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Reg. pons and medulla	Whole Blood	
							Before treatment	Before sacrifice
Rabbit 1	5x50 s.c.†	5	860	670	420	170		
Rabbit 2	5x50 "	5	650	450	430	190		
Rabbit 3	5x25 "	5	810	720	370	110	2200	2500
Rabbit 4	5x25 "	5	680	650	400	170		
Rabbit 5	5x25 "	5	560	470	310	90		
Rabbit 6	100 i.v.†	1	550	720			2200	2200
Rabbit 7	100 i.v.	1	690	970			3300	3300
Rabbits 1-7 Mean ± S.E.			690±40	660±70	390±20*	150±20	2600	2700
Control Mean ± S.E.			650±36 (31)	660±27 (25)	280±15 (22)	140±15 (20)		

* Significantly different from mean of control $P < 0.01$

† s.c.: subcutaneous
i.v.: intravenous

Table 33

Effect of histidine on the concentration of histamine in hypophysis and brain of rabbits pre-treated with iproniazid

Iproniazid (5 x 25 mg/Kg, subcutaneously, over 5 days) + Histidine (250 mg/Kg/2 hrs.) infused soon after the last injection of iproniazid.

Rabbits anaesthetized ½ hr. after the infusion. (No. of animals)

	Estimates expressed as ng/g of fresh tissue			
	Anterior lobe of hypophysis	Hypothalamus	Central grey matter	Reg. pons and medulla
abbit 1	1280	1390	680	350
abbit 2	540	1310	630	150
abbit 3	980	1340	450	190
abbits 1-3 Mean	930	1350	590	230
roniazid alone[1] 5 x 25-50 mg/Kg, .C., over 5 days) Mean ± S.E.	710 (5)	590 (5)	390 ± 20 (5)	150 ± 20 (5)
istidine alone[2] 250 mg/Kg/2 hrs.) ½ hr. Mean ± S.E.	830 ± 120 (5)	1360 ± 120 (5)	800 ± 74 (5)	280 ± 20 (5)
treated control Mean ± S.E.	650 ± 36 (31)	660 ± 27 (25)	280 ± 15 (22)	140 ± 15 (20)

[1]From Table 32

[2]From Table 18

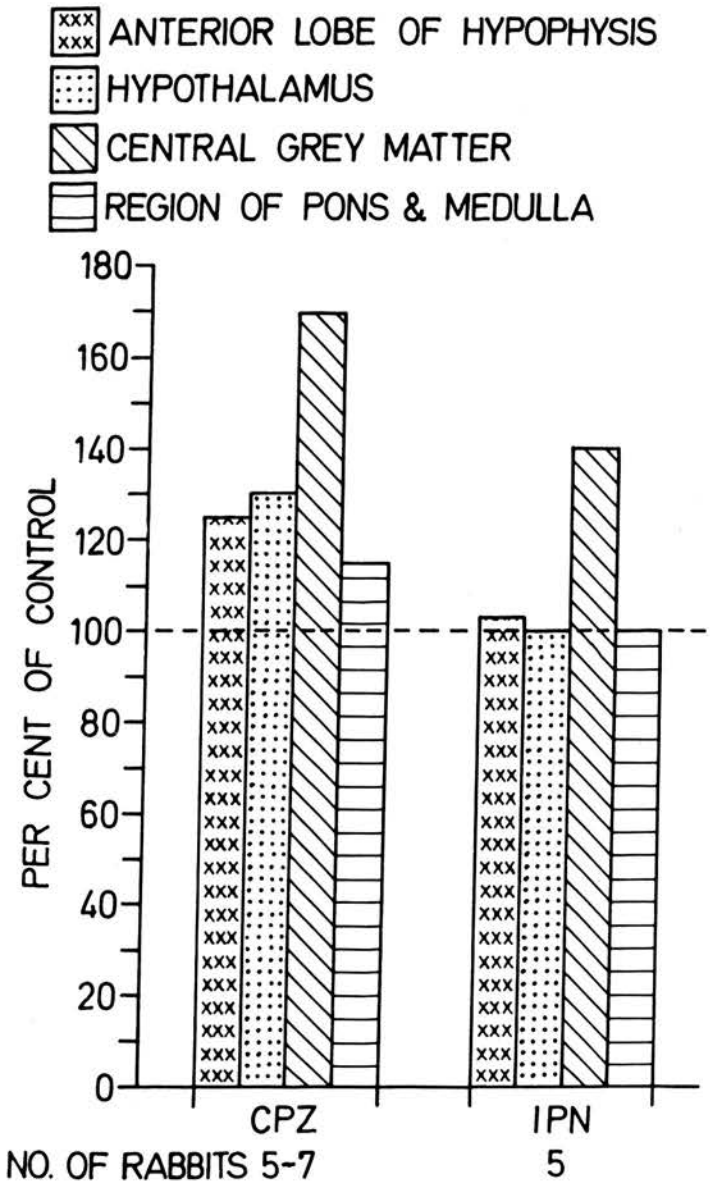


Fig. 10. Effect of drugs on the concentration of histamine in the anterior lobe of the hypophysis and different regions of the brain expressed as a percentage of the control. CPZ (Chlorpromazine, 3 x 10 mg/Kg i.v. over 24 hr); IPN (iproniazid, single i.v. injection of 100 mg/Kg over 24 hr or daily subcutaneous injection of 25 or 50 mg/Kg for 5 days).

IPN may, however, have acted in other ways (Pletscher, Gey and Burkard, 1966).

The finding that IPN did not raise the concentration of histamine in the hypothalamus, hindbrain or hypophysis may indicate that the turnover in these parts is slower than that in the midbrain.

The increase of monoamine concentration in rabbit brain due to injection of precursors, e.g. 5-HTP (Udenfriend et al, 1957) and tryptophan (Hess et al, 1959) is further enhanced by pre-treatment with IPN. In contrast, the present experiments (Table 33) showed that pre-treatment with IPN did not enhance the rise of histamine in brain produced by the infusion of histidine. Again, this finding suggests that the newly-formed histamine is probably not free.

SECTION 7/3

RESERPINE

The action of reserpine on stores of monoamines in brain and other tissues has been frequently reviewed (Shore, 1962; Pletscher, 1963; Carlsson, 1966).

Effect on monoamines. A single large dose of reserpine (5 mg/Kg i.v.) produces a rapid and marked reduction in the concentration of 5-HT in rabbit brain (Brodie, Pletscher and Shore, 1956; Hess, Shore and Brodie, 1956; Shore, Pletscher, Tomich, Carlsson, Kuntzman and Brodie, 1957; Pletscher, Shore and Brodie, 1956; Shore and Brodie 1957; Roos and Werdinius, 1962). A comparison of the dose-response relations and the time of recovery of noradrenaline and 5-HT in rabbit brain showed that the effects of reserpine on the two amines were very similar and that the curves were superimposable (Shore and Brodie, 1957). These authors suggested that 5-HT and noradrenaline are bound by a similar mechanism which is inactivated by reserpine, although the possibility was not ruled out that one amine could in turn effect the release of the other. Reserpine also decreases the concentration of dopamine in the corpus striatum of the rabbit brain (Anden, Roos and Werdinius, 1964).

Smaller doses of reserpine (0.1-0.5 mg/Kg) also produce a measurable effect on the concentration of monoamines in rabbit brain (Brodie et al, 1956; Shore and Brodie, 1957; Bertler, 1961).

Besides the depletion of monoamines, reserpine raises the concentration of 5-HIAA (Roos and Werdinius, 1962), dihydroxyphenylacetic acid and homovanillic acid (Anden et al, 1963) in rabbit brain.

Metabolism of reserpine and effect on behaviour. After the i.v. injection of labelled reserpine (5 mg/Kg) in rabbit, the drug readily entered the brain; the concentration was maximal within 15 min., and undetectable after 4 hr (Hess, Shore and Brodie, 1956). According to these authors the central action of reserpine, which lasted for about 48 hr, was related to the fall in the concentration of 5-HT in brain. Recently, however, it has been suggested that the intensity of the action is more closely related to the rate at which the amine is released (Brodie et al, 1966). This assumption was based on the following evidence: When 5-HTP was given to rabbits, the concentration of 5-HT rose significantly in the brain stem. In contrast, when 5-HTP was given 6 and 16 hr after treatment with reserpine, the effect was much smaller; at 36 hr, however, the brain was able to accumulate a much larger quantity of 5-HT. It was also found

that reserpine did not inhibit the formation of 5-HT *in vivo*. It was therefore concluded that recovery from sedation was closely related to the recovery of the uptake and storage of the amine.

Effect on histamine. Little is known about the action of reserpine on brain histamine. Various authors failed to demonstrate that reserpine had any significant effect on the concentration of histamine in brain of rat (Ungar and Witten, 1963), guinea pig (Waalkes et al, 1959), or rabbit (Waalkes et al, 1959; Burkhalter et al, 1960); these authors used chemical methods of assay. Adam and Hye (1966), however, employed biological methods and showed that reserpine reduced the concentration of histamine in cat's hypothalamus and thalamus, but not in hypophysis.

Reserpine releases histamine from rabbit blood *in vitro* and *in vivo* (Waalkes and Weissbach, 1956; Waalkes et al, 1959; Burkhalter et al, 1960) and is reported to deplete histamine from other tissues of the rat, without causing degranulation of the mast cells (Parrat and West, 1957).

It was desirable in the first instance to study the depletion and recovery of histamine in

blood, hypophysis and brain after treatment with reserpine. The investigations were further extended to study the effect of reserpine on the storage of histamine.

7/3/1 Materials and method

Reserpine base ('Serpasil', Ciba, pure substance) was dissolved in 20% ascorbic acid solution to give a concentration of 10 mg/ml. The solution was freshly prepared at the time of injection. A dose of 5 mg/Kg was injected into the marginal vein of the ear in all the experiments. Blood samples for haematocrit and histamine estimation were taken before treatment and just before bleeding the animal. The volume of blood collected from rabbits treated with reserpine was less than that from controls (mean 75 ml, range 40-100 (41)).

The experiments are described below:

(a) Depletion and recovery of histamine. Rabbits were injected with reserpine (5 mg/Kg i.v.) and sacrificed at various time intervals. Twenty-six rabbits were used.

(b) Effect of histidine after pre-treatment with reserpine. The object was to test the recovery of storage capacity of hypophysis, hypothalamus and platelets.

(1) Rabbits received reserpine (5 mg/Kg); 21.5 hr later they were infused with histidine (500 mg/Kg/2hr i.v.) and sacrificed about 30 min. after the end of infusion. Five rabbits were used.

(2) Rabbits received reserpine (5 mg/Kg); 45.5 hr later they were infused with histidine (500 mg/Kg/2hr) and sacrificed about 30 min after the end of infusion. Three rabbits were used.

(c) Effect of reserpine after pre-treatment with histidine. The aim was to test the effect of reserpine on newly-formed histamine. Rabbits were infused with histidine (500 mg/Kg/2 hr i.v.); 30 min after the end of infusion they were injected with reserpine (5 mg/Kg i.v.); animals were killed about 16 hr after the injection of reserpine. Five rabbits were used.

(d) Effect of histamine after pre-treatment with reserpine. The aim was to test for the uptake of histamine by the hypophysis, hypothalamus and blood in rabbits pre-treated with reserpine. Rabbits were injected with reserpine (5 mg/Kg i.v.); 21.5 hr later they were infused with histamine (2 mg/Kg/90 min i.v.) and sacrificed about 1 hr after the end of infusion. Two rabbits were used.

7/3/2 Results

Effect on behaviour. Rabbits treated with reserpine were kept at a temperature of about 21°C. Within 15-30 min after the injection, the rabbits were sedated; the respiration was rapid and laboured; the pupils were constricted; the nictitating membranes were relaxed and the eyes tightly closed. The animals assumed a sleeping posture and avoided light. Responses to sound and painful stimuli were diminished and in some instances the righting reflex was lost. About 4-5 hr after injection, the rectal temperature had fallen by 1-3°C and remained so for 16-24 hr. At about 48 hr most signs of sedation had disappeared. Nine rabbits out of a total of 50 died within the first 48 hr of treatment.

Infusion of histidine before or after the injection of reserpine had no apparent effect on behaviour.

One experiment was performed to test whether pre-treatment with iproniazid would counteract the effect of reserpine on histamine in brain. It was found that when reserpine (5 mg/Kg i.v.) was injected 6 hr after treatment with iproniazid

Table 34

Effect of single intravenous injection of reserpine (5 mg/Kg) on the concentration of histamine in hypophysis, brain and blood. Rabbits sacrificed 24 hours after treatment.

	Estimates expressed as ng/g of fresh tissue or ng/ml of whole blood							
	Anterior lobe of hypophysis	Hypothalamus	Medial thalamus	Central grey matter	Reg. pons and medulla	Cerebellum (vermis)	Whole Blood	
							before treatment	before sacrifice
Rabbit 1	200	150					3800	820
Rabbit 2	240	310					2700	540
Rabbit 3	270	260					5400	870
Rabbit 4	390	280					3000	540
Rabbit 5	270	320	120	170	80	70	7600	1400
Rabbit 6	200	270	150	140	70	70	4100	680
Rabbit 7	150	270	80	140		20		
Rabbits 1-7 Mean ± S.E.	250* ± 30	270* ± 20	120	150	80	50	4400±700	800*±130
Controls Mean ± S.E. (no. of rabbits)	650 ± 36 (31)	660 ± 27 (25)	270 ± 18 (19)	280±15 (22)	140 ± 15 (20)	60 ± 5 (12)		

* Significantly different from mean of control $P < 0.001$

(100 mg/Kg i.v.), rabbits showed signs of severe excitation similar to those seen during the infusion of L-DOPA (S. 6/2). The two rabbits died of hyperthermia 4 hr after injection of reserpine. Smaller doses of reserpine were not tried, since it was not known whether they would deplete the brain histamine.

Effect on histamine concentration. The effect of reserpine at 24 hr is shown in Table 34. Reserpine reduced the concentration of histamine in hypophysis, brain and blood. In the anterior lobe, hypothalamus and blood the concentration fell significantly ($P < 0.001$); in the hypophysis and hypothalamus it fell to about 40% of the control; in blood to 20%. The concentration in the thalamus, central grey and pons-medulla was also reduced. The concentration in the cerebellum, though normally very low (Table 12), remained unchanged.

The effect of reserpine at 16 hr is shown in Table 35. The concentration fell significantly ($P < 0.001$) to about 40% of the control in the anterior lobe and hypothalamus and to 25% in blood.

Table 35

Effect of single intravenous injection of reserpine (5 mg/Kg) on the concentration of histamine in hypophysis, hypothalamus and blood. (Rabbits sacrificed 16 hours after treatment

	Estimates expressed as ng/g or ng/ml			
	Anterior lobe of hypophysis	Hypothalamus	Whole blood	
			before treatment	before sacrifice
Rabbit 1	330	330	3800	1100
Rabbit 2	340	310	3500	1000
Rabbit 3	160	250	4900	820
Rabbit 4	200	260	3300	820
Rabbit 5	250	300	2200	540
Rabbit 1-5 Mean ± S.E.	260* ± 40	290* ± 20	3500±400	860*±100
Control Mean ± S.E. (No. of rabbits)	650 ± 36 (31)	660 ± 27 (25)		

* Significantly different from mean of control $P < 0.001$

(a) Depletion and recovery of histamine after treatment with reserpine (5 mg/Kg i.v.). Results are given in Table 36 and are shown graphically in Fig. 11.

In the anterior lobe of the hypophysis the reduction in the concentration of histamine was maximal at 16 hr (40% of the control) and the low concentration was maintained for 72 hr. At 96 hr the concentration had begun to rise.

The concentration in hypothalamus at 2-8 hr was 45-70% of the control. Maximal depletion occurred at 16-24 hr (40% of the control). At 48 hr the concentration began to recover and was about 60% of the control.

In blood the concentration was 70% of the control at 2 hr, 50% at 4 and 8 hr and 25% at 16 hr. Maximal reduction (20% of the control) was observed at 24 and 48 hr. At 72 hr the concentration rose to 45% of the control.

Recovery in all 3 areas was complete at one week.

(b) Effect of histidine after pre-treatment with reserpine.

(1) Table 37 and Fig. 12 show the effect of

Table 36

Depletion and recovery of histamine in the hypophysis, hypothalamus and blood after a single intravenous injection of reserpine (5 mg/Kg). Estimates of concentration expressed as ng/g of fresh tissue or ng/ml of whole blood.

	Controls Mean ± S.E.	Time interval in hours								
		2	4	8	16	24	48	72	96	168
Anterior lobe of hypophysis (no. of rabbits)	650 ± 36 (31)	850 850	390 650	800 510	330 340 160 200 250	200 240 270 390 270 200 150	200 300	240 240	630 390	590 650
Hypothalamus (no. of rabbits)	660 ± 27 (25)	310 380	510 470	360 250	330 310 250 260 300	150 310 260 280 320 270 270	400 350	480 390	310 330	760 570
Whole Blood before reserpine injection		3800 2200	4300 2700	4300 3000	3800 3500 4900 3300 2200	3800 2700 5400 3000 7600 4100	3300 4500	2700 5400	2100 5400	5300 3500
Whole Blood before sacrifice		2800 1500	2400 1200	2200 1600	1100 1000 820 820 540	820 540 870 540 1400 680	380 1140	1400 2200	2600 3800	4500 4300

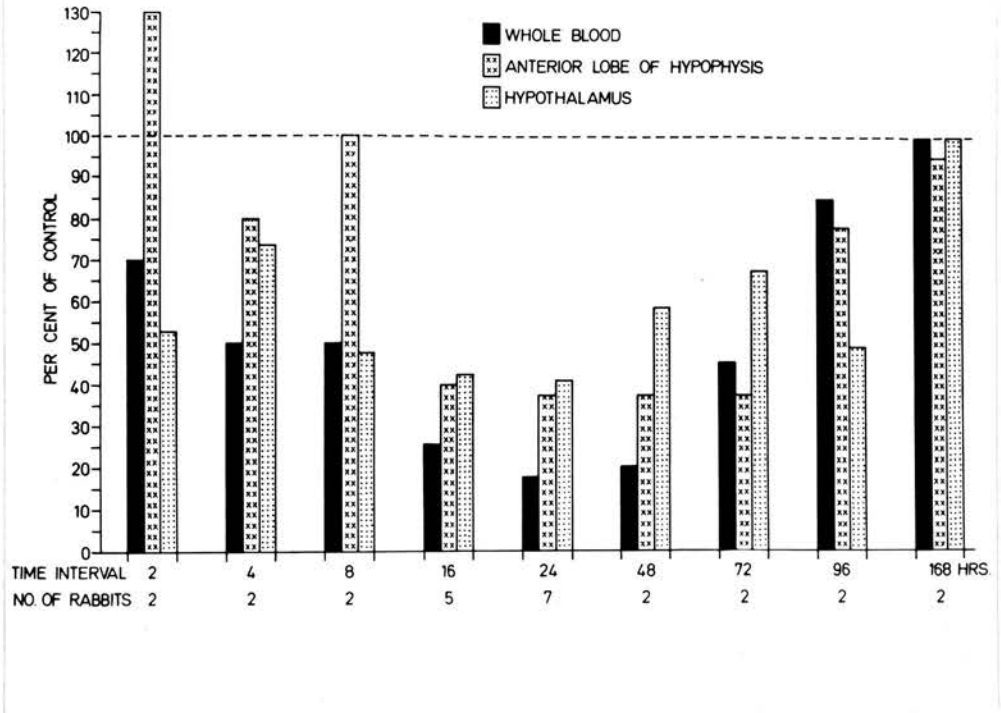


Fig. 11. Depletion and recovery of histamine in rabbit's whole blood, anterior lobe of hypophysis and hypothalamus, after a single i.v. injection of reserpine (5 mg/Kg). The horizontal line at 100 per cent represents the mean control value for the concentration of histamine. The columns represent the mean concentrations of histamine expressed as a percentage of the control in the 3 regions at different time intervals after the injection of reserpine.

i.v. infusion of histidine on the concentration of histamine in hypophysis, hypothalamus and blood in rabbits pre-treated with reserpine 21.5 hr earlier.

In the anterior lobe, the mean concentration rose from 40 to 110% of the control (not treated with any drug); in the hypothalamus from 40 to 80%. In blood, however, the concentration remained unchanged (15% of the control); the infusion of histidine did not appear to restore the amine depleted by reserpine.

(2) Table 38 and Fig. 12 show the effect of i.v. infusion of histidine in rabbits pre-treated with reserpine 45.5 hr earlier.

In the anterior lobe, the concentration rose from 40 to 110% of the control; in the hypothalamus, from 55 to 155%; in blood, the concentration remained unchanged (15% of the control).

(c) Effect of reserpine after pre-treatment with histidine. Table 39 and Fig. 13 show the effect of reserpine injected 30 min after the end of histidine infusion; rabbits were killed 16 hr after the injection of reserpine.

The concentration of histamine fell in hypophysis, hypothalamus and blood to the same extent as with reserpine alone. It seems, therefore, that

Table 37

Effect of histidine on the concentration of histamine in hypophysis, hypothalamus and blood of rabbits pre-treated with reserpine.

(No. of animals)

	Estimates expressed as ng/g or ng/ml			
	Anterior lobe of hypophysis	Hypothalamus	Whole blood	
			Before treatment	Before sacrifice
Untreated controls Mean ± S.E.	650 ± 36 (31)	660 ± 27 (25)		
Reserpine alone[1] (5 mg/kg i.v.) at 24 hr Mean ± S.E.	250 ± 30 (7)	270 ± 20 (7)	4400 ± 700 (6)	800 ± 130 (6)
Histidine alone[2] (500 mg/kg/2 hr) at ½ hr Mean ± S.E.	800 ± 80 (11)	1480 ± 130 (5)	3300 ± 400 (10)	2900 ± 460 (10)
Reserpine (5 mg/kg i.v.) + Histidine (500 mg/kg/2 hr) infused i.v. 21.5 hr after the injection of reserpine. Rabbits sacrificed about ½ hr after the end of histidine infusion.				
Rabbit 1	860	630	4300	650
Rabbit 2	280	440	4900	330
Rabbit 3	580	440	6500	1090
Rabbit 4	1380	560	4300	1090
Rabbit 5	550	660	2800	680
Rabbits 1 - 5 Mean ± S.E.	730* ± 190	550+ ± 50	4600 ± 600	800■ ±200

[1] From Table 34, p. 151a

[2] From Table 18, p. 100a

* Significantly different from reserpine-treated $P < 0.02$; but not different from histidine-treated or untreated control

+ Significantly different from reserpine-treated $P < 0.001$

■ Significantly different from pre-injection control $P < 0.001$

Table 38

Effect of histidine on the concentration of histamine in hypophysis, hypothalamus and blood of rabbits pre-treated with reserpine. (No. of animals)

	Estimates expressed as ng/g or ng/ml			
	Anterior lobe of hypophysis	Hypothalamus	Whole Blood	
			before treatment	before sacrifice
Untreated controls Mean ± S.E.	650 ± 36 (31)	660 ± 27 (25)		
Reserpine alone[1] (5 mg/Kg i.v.) at 48 hr Mean	250 (2)	380 (2)	3900 (2)	760 (2)
Histidine alone[2] (500 mg/Kg/2 hr) at ½ hr Mean ± S.E.	800 ± 80 (11)	1480 ± 130 (5)	3300±400 (10)	2900±460 (10)
Reserpine (5 mg/Kg i.v.) + Histidine (500 mg/Kg/2 hr) infused i.v. 45.5 hr after the injection of reserpine; rabbits sacrificed ½ hr after end of infusion				
Rabbit 1	710	940	3300	490
Rabbit 2	420	1150	3300	650
Rabbit 3	1000	1000	3300	490
Rabbits 1-3 Mean	710 (3)	1030 (3)	3300 (3)	540 (3)

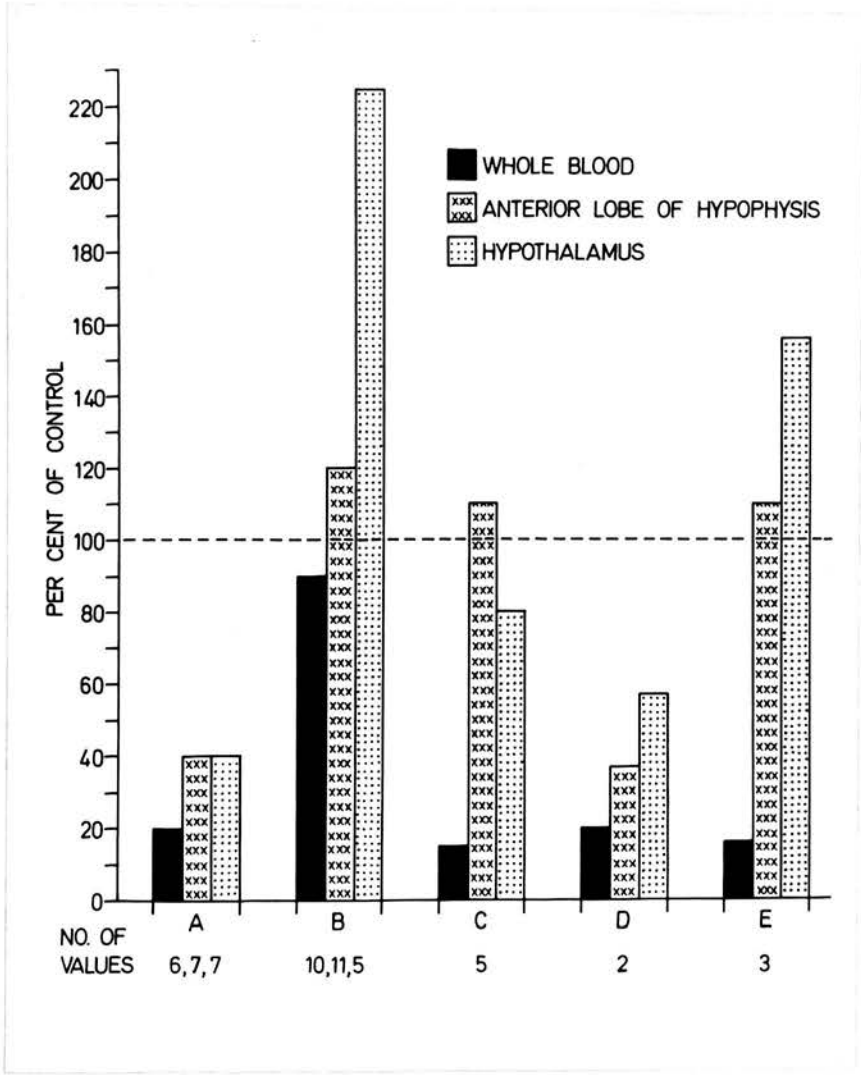


Fig. 12. Effect of histidine in rabbits pretreated with reserpine. The horizontal line at 100 per cent represents the mean control value for the concentration of histamine in whole blood, anterior lobe of hypophysis and hypothalamus. The columns represent the mean concentration of histamine in the 3 regions expressed as a percentage of the control after treatment. EFFECT OF :
A: reserpine (5 mg/Kg i.v.) at 24 hr after the injection.
B: histidine (500 mg/Kg/2 hr) at 30 min after the end of infusion.
C: reserpine (5 mg/Kg i.v.) followed 21.5 hr later by an infusion of histidine (500 mg/Kg/2 hr); rabbits were anaesthetized at 30 min after the end of infusion.
D: reserpine (5 mg/Kg i.v.) at 48 hr after the injection.
E: reserpine (5 mg/Kg i.v.) followed 45.5 hr later by an infusion of histidine (500 mg/Kg/2 hr); rabbits were anaesthetized 30 min after the end of infusion.

all the newly-formed histamine was depleted by reserpine.

(d) Effect of histamine after pre-treatment with reserpine. Table 40 shows that in rabbits pre-treated with reserpine, the anterior lobe takes up exogenous histamine; neither the hypothalamus nor blood took up histamine in significant amounts.

7/3/3 Discussion

Depletion. Evidence has already been presented for the independence of the concentration of histamine in blood and that in the hypophysis and brain (S. 4/3, 5/5). In the present experiments, it might appear that the fall in the concentration of histamine in brain and hypophysis was related to the fall in blood. The following observations suggest that although the two events are concurrent, they are not causally related:

(1) Whereas the concentration in hypothalamus and hypophysis was reduced by reserpine to 40% of the control, that of the blood was reduced to 20%, (Table 34).

(2) At the time when histamine in blood was reduced to about 20% of the control (not treated with any drug), the concen-

Table 39

Effect of reserpine on the concentration of histamine in hypophysis, hypothalamus and blood of rabbits pre-treated with histidine. (No. of animals)

	Estimates expressed as ng/g or ng/ml			
	Anterior lobe of hypophysis	Hypothalamus	Whole blood	
			Before treatment	Before sacrifice
treated controls an ± S.E.	650 ± 36 (31)	660 ± 27 (25)		
stidine alone[1] 00 mg/Kg/2 hr.) at ½ hr. an ± S.E.	800 ± 80 (11)	1480 ± 130 (5)	3300 ± 400 (10)	2900 ± 460 (10)
stidine alone[2] 00 mg/Kg/2 hr.) at 16hrs. an	540 (3)	1340 (3)	3000 (3)	3100 (3)
serpine alone[3] mg/Kg i.v.) at 16 hrs. an ± S.E.	260 ± 40 (5)	290 ± 20 (5)	3500 ± 400 (5)	860 ± 100 (5)
stidine (500 mg/Kg/2 hr.) + serpine (5 mg/Kg i.v.) jected 30 min. after the fusion; rabbits sacri- ced at 16 hrs. after the jection of reserpine				
Rabbit 1	250	420	3300	820
Rabbit 2	240	450	2700	680
Rabbit 3	230	390	8700	2700
Rabbit 4	210	270	8700	1600
Rabbit 5	210	270	5400	1200
bbits 1-5 an ± S.E.	† 230 ± 10	† 360 ± 40	5800 ± 1300	* 1400 ± 400

[1] From Table 18 p. 100a
[2] From Table 20 p. 104a
[3] From Table 35 p. 152a

* Significantly different from pre-treated control $P < 0.001$

† Significantly different from untreated controls $P < 0.001$; but not different from values obtained after reserpine alone (5 mg/Kg i.v., at 16 hrs.)

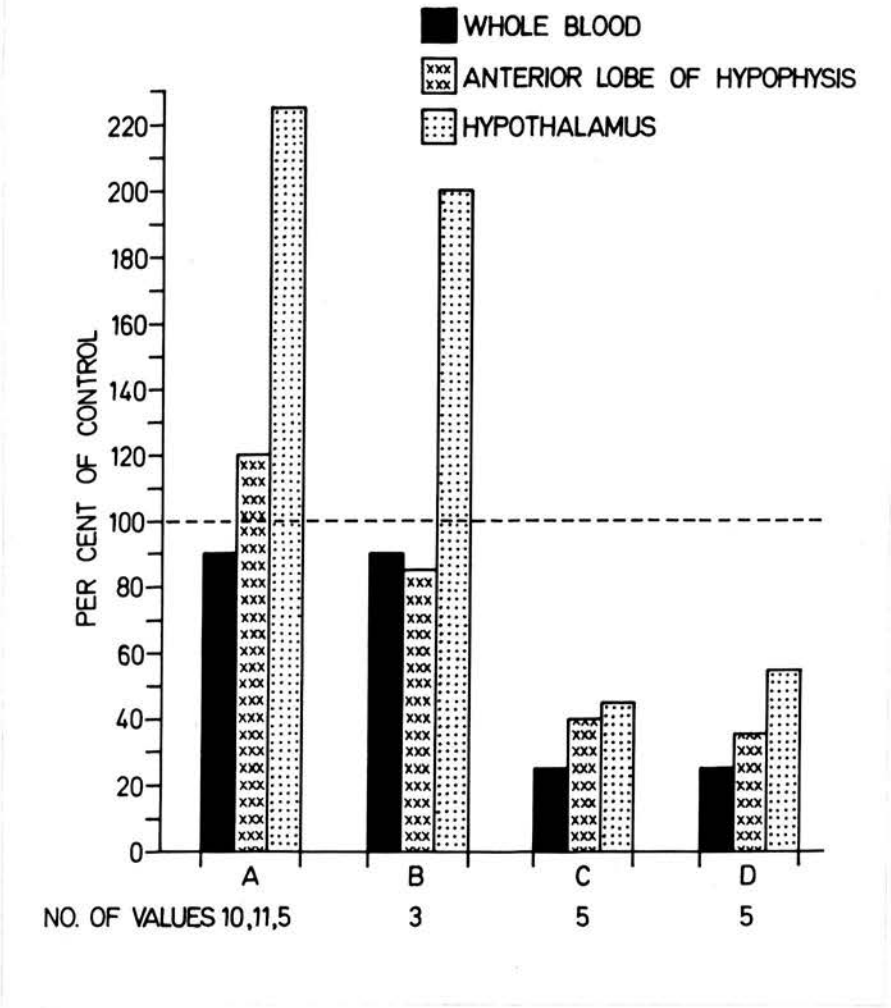


Fig. 13. Effect of reserpine in rabbits pre-treated with histidine. The horizontal line at 100 per cent represents the mean control value for the concentration of histamine in whole blood, anterior lobe of hypophysis and hypothalamus. The columns represent the mean concentration in the 3 regions expressed as a percentage of the control after treatment. EFFECT OF :
A: histidine (500 mg/Kg/2 hr) at 30 min after the end of infusion.
B: histidine (500 mg/Kg/2 hr) at 16 hr after the end of infusion.
C: reserpine (5 mg/Kg i.v.) at 16 hr after the injection.
D: histidine (500 mg/Kg/2 hr) followed 30 min later by reserpine (5 mg/Kg i.v.); rabbits were sacrificed 16 hr after the injection of reserpine.

tration in the hypophysis was 110% and in the hypothalamus 80 and 155% (Fig. 12, C and E).

(3) Intravenous injection of glycogen reduced the concentration in blood, but not in the hypophysis or brain (Fig. 14).

(4) Reserpine lowered the concentration of histamine in blood but not in the cerebellum (Table 34).

Others have failed to detect any significant change in the concentration of histamine in rabbit brain after treatment with reserpine (S. 7/3). The present results for the hypothalamus agree with those reported for the cat (Adam and Hye, 1966). The failure of reserpine to release histamine from the hypophysis of cat (Adam and Hye, 1966) can be related to the finding that reserpine does not release histamine from mast cells (Parrat and West, 1957). In the rabbit, however, reserpine reduced the concentration in the anterior lobe (Fig. 11), probably because the amine is of non-mast origin; rabbit tissues are known to contain few mast cells (Constantinides, 1953).

Reserpine releases histamine from rabbit platelets *in vitro* and *in vivo* (S. 7/3). The rate of histamine depletion in blood (Fig. 11) is

similar to that reported by Waalkes and Weissbach (1956). Since reserpine does not cause a reduction in the concentration of circulating platelets (Shore et al, 1956), the decline of histamine in blood therefore represents a true release.

The same dose of reserpine (5 mg/Kg i.v.) lowered the concentration of monoamines in rabbit brain to 25% of the control within 30 min. and to 10% within 4 hr (Brodie et al, 1956; Shore and Brodie, 1957). By comparison, the present results show that reserpine does not deplete histamine in brain to the same extent or as rapidly as it depletes the monoamines.

It has been suggested that reserpine lowers the monoamine concentration in brain by blocking a mechanism which pumps the amines across a membrane into particles (Pletscher, Shore and Brodie, 1956; Brodie and Shore, 1957). According to this hypothesis the depletion of amines is pictured as a consequence of passive leakage of the amines from their stores, and the low concentration is a balance between synthesis and rapid catabolism. Giarman (1961) found that more 5-HT was present in the supernatant fraction than in the particulate

Table 40

Uptake of histamine by hypophysis, hypothalamus and blood in rabbits pre-treated with reserpine (5mg/Kg i.v.). Histamine (2 mg/Kg/90 min.) infused 21.5 hr. after the injection of reserpine. (No. of rabbits).

	Estimates of concentration as ng/g or ng/ml			
	Anterior lobe of hypophysis	Hypothalamus	Whole Blood	
			before treatment	before sacrifice
Rabbit 1	680	230	3800	970
Rabbit 2	1260	280	5300	1200
Reserpine, 5mg/Kg at 24 hrs[1] Mean ± S.E.	250±30 (7)	270±20 (7)	4400±700 (6)	800±130 (6)
Histamine, 5mg/Kg infused over 2 hr.[2] Mean ± S.E.	5040±370 (5)	690±110	3100 (3)	3800 (3)
Untreated controls Mean ± S.E.	650±36 (31)	660±27 (25)		

[1] taken from table 34, page 151a

[2] taken from table 19, page 102a

fraction of the rat brain after treatment with reserpine. Hence reserpine might have acted in a similar way on the storage mechanism of histamine in brain, hypophysis and platelets. The difference in the intensity of action of reserpine on brain histamine and monoamines could be related to the different rates of synthesis and catabolism of the amines.

Recovery. The concentration of histamine in blood, hypophysis and hypothalamus remained low for several days, even when it could be expected that reserpine had disappeared from brain and whole body (Hess et al, 1956). Recovery was complete in 7 days (Fig. 11). The present results agree with those reported for the recovery of histamine in the cat's hypothalamus (Adam and Hye, 1966), and for the recovery of 5-HT and noradrenaline in rabbit brain (Brodie et al, 1956; Shore and Brodie, 1957).

Recovery of histamine in blood was similar to that reported by Waalkes and Weissbach (1956) for 5-HT and histamine. The rate of recovery of histamine in blood, hypophysis and brain was roughly the same, which might suggest that histamine in the 3 sites is bound by a similar mechanism.

Administration of histidine to rabbits pre-treated with reserpine (Tables 37 and 38. Fig. 12).

(a) Effect on hypothalamus. Reserpine did not prevent the formation of histamine from exogenous histidine, since some of the amine already depleted by reserpine was partially or completely replenished. By comparison, reserpine did not inhibit 5-HTP DC in rabbit brain in vivo (Shore et al, 1957) nor did it interfere with the formation of 5-HT from exogenous 5-HTP (Shore et al, 1957; Brodie et al, 1966).

When histidine was infused 21.5 hr after the injection of reserpine, the concentration rose from 40 to 80% of the control. But when histidine was infused 45.5 hr after reserpine, the concentration rose from 55 to 155% of the control. The infusion of histidine alone, however, raised the concentration to 225% of the control. These findings indicate that although decarboxylation of histidine was not affected, the storage capacity was impaired at 24 hr after the injection of reserpine; at 48 hr the storage capacity had already recovered but was not yet complete.

These results are comparable with those

reported by Brodie et al (1966) who found that the ability of rabbit brain to store 5-HT (from exogenous 5-HTP) began to recover at 36 hr after the injection of reserpine; these authors concluded that recovery from sedation was related to the recovery of storage capacity for 5-HT. Hence the theory for the role of 5-HT in the central action of reserpine (S. 7/3) could also hold true for histamine. In the present experiments rabbits recovered from sedation at the time when the low concentration of histamine in the hypothalamus had begun to rise and when the hypothalamus was able to hold larger amounts of newly-formed histamine.

(b) Effect on hypophysis. Reserpine alone (5 mg/Kg) reduced the concentration in the anterior lobe to about 40% (at 24 and 48 hr) (Table 36 and Fig. 11). Infusion of histidine alone (500 mg/Kg/2 hr) did not raise the concentration of histamine significantly (Table 18). But when the same dose of histidine was infused 21.5 and 45.5 hr after the injection of reserpine, the concentration rose from 40 to 110% of the control (Fig. 12). This rise is difficult to explain. However, two

possibilities can be considered: (a) It is possible that when the stores of histamine are depleted, as by reserpine, the hypophysis is able to form histamine from exogenous histidine and retain it, (b) It is probable that when histidine was infused in reserpinized rabbits, histamine may have been formed in blood or released from tissues and then taken up by the hypophysis. To test this assumption, rabbits were treated with reserpine and 21.5 hr later they were infused with histamine (Table 40). The anterior lobe was found to take up histamine under these conditions.

(c) Effect on blood. Reserpine alone (5 mg/Kg) reduced the concentration of histamine to 20% of the control at 24 and 48 hr (Table 36, Fig. 11). Infusion of histidine alone did not raise the concentration of histamine (Fig. 6). When histidine was infused 21.5 and 45.5 hr after the injection of reserpine, the concentration was still about 20% of the control, indicating that the capacity of platelets to store histamine was still impaired.

Administration of reserpine to rabbits pre-treated with histidine.

(a) Effect on hypothalamus. The infusion of histidine (500 mg/Kg/2 hr) raised the concentration of histamine to 225% of the control at ½ hr and to 200% at 16 hr. Reserpine alone lowered the concentration to 45% at 16 hr. When reserpine was injected 30 min after the infusion of histidine and rabbits were killed 16 hr after the injection of reserpine, the concentration fell from 225 to 55% of the control (Table 39, Fig. 13). It seems therefore, that reserpine released all the newly-formed histamine. This finding supports the hypothesis (S. 5/5) that the newly-formed histamine is probably bound.

(b) Effect on hypophysis and blood. When reserpine was injected 30 min after the infusion of histidine, the concentration fell to the same extent as with reserpine alone.

Uptake of histamine by hypothalamus, hypophysis and blood in rabbits pre-treated with reserpine. (Table 40). Only the anterior lobe of the hypophysis took up some exogenous histamine. This finding indicates that even though reserpine impairs the capacity of the hypophysis to store the amine, it did not prevent uptake.

SECTION 8

GENERAL DISCUSSION ON THE EFFECT OF AMINO ACIDS AND DRUGS

Since histamine is unevenly distributed in the brain (Table 12), the study of its concentration on a regional basis has made it possible to detect changes after treatment with amino acids and drugs. The effects were more prominent in the midbrain and hypothalamus than elsewhere in the brain.

8/1 BLOOD

The concentration of histamine in whole blood of untreated rabbits was high and varied over a wide range (Table 15). Histamine was estimated in blood as a control, since changes in blood histamine might have accounted for the changes found in brain. However, evidence presented in Sections 4/3, 5/5 and 7/3/3 indicates (a) that histamine extractable from brain and hypophysis derives mainly from the tissue and not from blood and (b) that changes in the histamine concentration in these tissues after treatment with drugs are independent of changes in the blood. Treatment with histidine, histamine, α-methyldopa, DOPA,

5-HTP or tryptophan did not alter significantly the concentration of histamine in blood. Treatment with reserpine produced a marked reduction.

8/2 HYPOPHYSIS

The concentration of histamine in rabbit's hypophysis (Table 12) was lower and less variable than that of the dog and cat (Table 2) which may be explained by the absence of mast cells; experiments with reserpine (Table 36) are consistent with this view.

None of the amino acids used produced a detectable change in the concentration of histamine extractable from the anterior lobe. The infusion of histamine, however, raised the concentration in both lobes of the hypophysis (Table 19). These findings confirm the view that the gland is outside the 'blood-brain barrier' (Davson, 1956). The concentration of histamine in the anterior lobe remained unchanged after treatment with chlorpromazine or iproniazid. After treatment with reserpine, the concentration fell to the same extent as in the hypothalamus.

8/3 BRAIN

The effect of amino acids and drugs was studied mainly on the concentration of histamine in the hypothalamus and midbrain.

(1) Effect of amino acids. The infusion of histidine led to a rise of histamine in many parts of the brain; the rise was greatest in the midbrain and hypothalamus and depended on the dose (Fig. 6). The following observations suggest that histamine was formed in the brain by local decarboxylation of histidine. First, after treatment with histidine the concentration of the free amino acid was found to be high in brain (Table 46), indicating that like other amino acids (Lajtha and Mela, 1961) it readily entered the brain; secondly, after the infusion of a large dose of histamine the concentration did not rise in the hypothalamus or thalamus (Table 19).

The infusion of large doses of histidine did not appear to affect the behaviour of the rabbit (S. 5/5), which might suggest that histamine formed in brain was probably 'bound'. The following evidence supports this assumption (a) newly-formed histamine disappeared slowly from the brain

(Fig. 8); (b) reserpine released all the newly-formed histamine (Fig. 13) and (c) chlorpromazine and iproniazid, which are known to inhibit the enzymes that catabolize histamine (S. 7/1 and 7/2), did not potentiate the effect of histidine on the concentration of histamine (Tables 31 and 33).

Treatment with α-methyldopa, DOPA or 5-HTP increased the concentration of histamine in the midbrain (Fig. 9). Simultaneous infusion of each of these amino acids with histidine did not interfere with the formation of histamine. These findings suggest that α-methyldopa does not inhibit the DC for histidine or that a specific DC for histidine exists in rabbit brain.

These results are difficult to interpret without further information. It would be desirable to estimate histidine and 1,4-methyl-histamine in brain and to study the properties of DC for histidine and the localization of histamine in brain.

(2) Effect of drugs. Chlorpromazine and iproniazid raised the concentration of histamine in the midbrain probably by inhibiting the catabolism of histamine.

Reserpine released histamine from different parts of the brain but did not prevent the formation of histamine from exogenous histidine. The capacity of hypothalamus to store the amine was greatly impaired at 24 hr but had partly recovered at 48 hr (Fig. 12). The recovery of histamine concentration and the binding mechanism in the hypothalamus coincided with the recovery of the rabbit from sedation; these observations might suggest that histamine was implicated in the central actions of reserpine. These findings are similar to those reported for 5-HT in the rabbit brain, (Brodie et al, 1966).

Since treatment with reserpine caused a simultaneous depletion of histamine in brain, hypophysis and blood, it may be that histamine is bound by a similar mechanism at the three sites.

The present work shows that the concentration of histamine in brain, particularly in the hypothalamus and midbrain, can be altered by several amino acids and drugs. The results do not indicate in any way the function of histamine in brain but suggest a relationship with other pharmacologically active amines.

APPENDIX 1

STUDIES IN THE CAT

The first experiments with histidine were carried out in the cat as an extension of an earlier investigation by Adam and Hye (1966).

1/1 Materials and method

Cats were anaesthetized with pentobarbitone-sodium (45 mg/Kg i.p.) or chloralose (80 mg/Kg i.v. after induction with ether) and then infused with saline (i.v. through the femoral vein or intra-arterially through the carotid) as a control for the infusion with histidine solution. The method of removal and dissection of cat's brain was similar to that described by Hye (1964). Extraction and purification of histamine is described in S. 2/9. The conditions of the present experiments differed slightly from those of Adam and Hye (1966), these workers anaesthetized the cat with ether and estimated histamine in different parts of the hypophysis and hypothalamus. In the present work histamine was estimated in the whole hypophysis, one half of the hypothalamus, the medial thalamus and the cerebral cortex.

Preparation of the histidine solution has been described in S. 2/3. Cats were anaesthetized and then infused with histidine (500 mg/Kg/2 hr i.v. or intra-arterially).

1/2 Results

(a) Concentration of histamine in hypophysis and brain (Table 41). The mean concentrations are compared with those obtained by Adam and Hye (1966). The present results in the brain are slightly higher than those obtained when ether was used as an anaesthetic (Adam and Hye, 1966). It is not clear how the anaesthetic affects the concentration of histamine in cat's brain.

(b) Effect of histidine on the concentration of histamine (Table 42). The mean concentrations in the hypophysis, hypothalamus and cerebral cortex were not significantly different from those of the saline-infused controls. In the medial thalamus, the mean concentration rose significantly to about 135% of the control ($P < 0.01$).

The same dose of histidine (500 mg/Kg/2 hr) raised the concentration of histamine in brain, both in the conscious and anaesthetized rabbit (S. 5). The negative results in the cat are difficult to

Table 41

Histamine in cat's hypophysis and brain. Estimates of concentration as nanograms per gram of fresh tissue.

	Anaesthetic mg/Kg	Infusion of saline, 16.7ml/Kg/2hr	Region			
			Whole hypophysis	Hypothalamus	Medial thalamus	Cerebral cortex
Cat 1	pentobarbitone, 45	intraven.	1270	900	310	110
Cat 2	" 45	"	3620	730	400	70
Cat 3	" 45	intra-art.	1800	1440	470	170
Cat 4	" 45	"	1760	1070	380	150
Cat 5	chloralose 80		2570	880	390	60
Cat 6	" 80		2420	1230	310	110
Cat 7	pentobarbitone, 45	intraven.	2160*	720*	340	
Cats 1-7 Mean ± S.E.			2230±290	1000±100	370±20	110±20
Controls from Adam and Hye, 1966 Mean, range, (no.)	Ether		2410* 780-5660 (25)	690* 370-1120 (15)	250 110-410 (17)	90 40-130 (8)

* Pooled value from different parts.
Hypophysis = anterior lobe + posterior lobe + infundibulum
Hypothalamus = ventral hypothalamus + dorsal hypothalamus + corpora mamillaria + pre-optic region

interpret, but could be due to the effect of anaesthetic, species difference, differences in turnover rates of the amine or some other factors.

Table 42

Effect of histidine infusion (500 mg/Kg/2hr.) on the concentration of histamine in hypophysis and brain of cats anaesthetized with pentobarbitone. Estimates expressed as nanograms per gram of fresh tissue. (No. of cats)

	Route of histidine infusion	Region			
		Whole hypophysis	Hypothalamus	Medial thalamus	Cerebral cortex
Cat 1	intraven.	1620	1110	500	150
Cat 2	intraven.	1740	1130	410	110
Cat 3	intra-art.	3350	1250	610	120
Cat 4	intra-art.	1990,	1280,	530	190
Cat 5	intraven.	1510†	1490†	510	
Cats 1-5 Mean ± S.E.		2040±340	1250±70	510±30*	140
Controls mean ± S.E. (from Table41)		2230±290 (7)	1000±100 (7)	370±20 (7)	110±20 (6)

* Significantly different from mean of control $P < 0.01$

† Pooled value from various parts.

APPENDIX 2

ESTIMATION OF HISTIDINE

2/1 Introduction

The rise of histamine in brain after the i.v. infusion of histidine raised the question whether histamine was formed during the extraction procedure. That histamine may be formed from histidine in the process of extraction has been shown to occur. Non-enzymatic decarboxylation of histidine had to be excluded before it could be assumed that histamine extractable from brain after the infusion of histidine was formed endogenously. This was especially necessary since after the infusion of large amounts of histidine, the concentration of this amino acid was high in the plasma and brain (Table 46).

Although the method described by Barsoum and Gaddum (1935) and later modified by Code (1937a) has been used for the extraction of histamine from tissues, some have criticized it. Heating of the extract in strong HCl to destroy other pharmacologically active substances, may lead to the formation of histamine by spontaneous decarboxylation of histidine. As early as 1911, Ewins and Pyman

found that histamine can be formed from histidine when the latter is heated in concentrated HCl at high temperatures. Barsoum and Gaddum (1935) warned against desiccation when heating the tissue extract in HCl. Later Åkerblom (1941) found that histamine was formed when histidine was extracted by Code's method (1937a). These findings were confirmed by Schmiterlow (1949). Hughes, Salvin and Wood (1951) concluded that trichloracetic acid might be responsible for the formation of histamine. These findings, however, were not confirmed by others (Emmelin, Kahlson and Wicksell, 1941; Emmelin, 1945).

The following experiments were carried out to test for the possible formation of histamine from histidine under the conditions of our method which includes extraction in TCA and heating in 6 N HCl at 98-100°C.

2/2 Estimation of histidine in pure solutions

The colorimetric method of MacPherson (1946) was used.

2/2/1 Special Equipment

Beckman DB Spectrophotometer and Unicam SP. 1300 Colorimeter.

2/2/2 Chemicals and solutions

All chemicals used were of analytical grade:
N/1 HCl, made up from an ampoule (concentration 36.46% w/v, BDH)
1% (w/v) Sulphanilic acid solution in N/1 HCl
5% (w/v) Sodium nitrite, prepared fresh for each experiment
20% (w/v) Sodium carbonate
20% (w/v) Ethanol (re-distilled twice).
Histidine solution: concentrated stock solution (4 mg base/ml water) was kept at 4°C; histidine standards were prepared fresh from this stock.

2/2/3 Procedure

(a) Pipette histidine solution into a stoppered 25 ml volumetric flask and make up to 5 ml with water.

(b) Add 1 ml of 1% sulphanilic acid solution and add 1 ml of 5% $NaNO_2$. Mix and stand for 5 min using a stop watch, shaking the flask occasionally.

(c) Blow in from a rapid delivery pipette, 3 ml of 20% Na_2CO_3. Shake for about 10 seconds and

(d) add 10 ml of 20% ethanol. Mix while cooling under the tap.

(e) Make to volume (25 ml) with water and read in the colorimeter.

To prepare a blank, the same procedure was repeated starting with 5 ml water or 5 ml buffer (100 mEq/l Na^+).

Chemical assay. The Beckman Spectrophotometer was used to determine the wave-length for peak absorbance of the coloured substance which was found to be between 490 and 510 mμ. The Unicam colorimeter was subsequently used for the estimation of histidine; the filter was No. 2 Ilford 622, bright spectrum blue (375-535 mμ). The two instruments gave essentially the same readings.

Calibration curve. Histidine standards, 20, 40, 60 and 80 μg were used, since the colorimeter is most sensitive in the absorbance range 0 to 0.3. Standards of histidine dissolved in water or buffer (100 mEq/l Na^+) gave similar readings. The calibration curve was linear in this range and was tested in each experiment.

2/3 Estimation of histidine in brain and blood plasma.

2/3/1 Special Equipment.

Automatic Amino Acid Analyser (Evans Electroselenium).

2/3/2 Chemicals and solutions

All chemicals used were of analytical grade:
Anionic resin (Dowex-2-Chloride x 8, 100-200 mesh)
N/1 HCl
0.02 N HCl (prepared from N/1 HCl)
1% picric acid solution in water (w/v), prepared from 50% solution (BDH).

2/3/3 Procedure

Preparation of the column. The resin (Dowex-2-chloride x 8) was washed 3 times in N/1 HCl and steeped in the same acid and kept at 4°C. The resin was then poured into a tube (9 mm diameter) the lower end of which was closed by a disc of sintered glass. The column was 2 cm high with volume of 10.2 cm^3. The column was then washed with distilled water until the effluent became nearly neutral; the pH was measured electrometrically. Time required for wash was 50 min. When the pH was close to that of distilled water (5.2), a round filter paper (Whatman No. 1) was cut and applied to the top of the resin to prevent disturbance. The column was now ready for application of the brain or plasma extract.

The flow rate was not critical (about 25 ml/ 15 min.). Picric acid and all acidic amino acids were adsorbed on the column, but some of the basic amino acids may also have been adsorbed. The basic amino acids, including histidine, were eluted with 4 x 5 ml aliquots of 0.02 N HCl; the acidic acids remained adsorbed. The effluent as well as the eluate was collected in a 50 ml measuring cylinder and then transferred to a 500 ml round-bottomed glass-stoppered flask for freeze-drying. The residue was taken up in 0.1 N HCl and applied to a column in the Automatic Analyser.

Extraction of blood plasma. One rabbit received 3 infusions of histidine (3 x 500 mg/Kg i.v. over 24 hr, S. 5/1). Thirty min after the end of last infusion, it was anaesthetized and heparinized; the carotid arteries were cannulated and 10 ml of blood was collected and centrifuged at 2500 r.p.m. for 30 min at 4°C. A two ml aliquot of plasma was transferred to a large centrifuge tube to which 16 ml of 1% picric acid was added (8 volumes) and mixed with a glass rod. This suspension was centrifuged at 2500 r.p.m. for 30 min at 4°C. An aliquot of supernatant (16 ml) was

decanted into a measuring cylinder and applied to the Dowex-2 column, as described. The final volume of the effluent was 35.5 ml.

Extraction of brain. After bleeding the rabbit, the head was perfused with Ringer-Locke solution. The brain was then removed and divided in the sagittal plane; one half (5 g) was placed in a large homogenizer (immersed in ice, 4°C) and homogenized with water (2.5 ml of water/g of brain). The pestle was washed with water and the final volume was made up to 20 ml. A 2 ml aliquot of brain homogenate (equivalent to 500 mg of tissue) was transferred to another homogenizer. To this homogenate 16 ml of 1% picric acid (8 volumes) was added and the mixture homogenized again. The final suspension was transferred (by teat pipette) to 2 graduated 10 ml centrifuge tubes (9 ml in each) and both were centrifuged, as above, until the supernatant was clear. The supernatant was decanted into a 25 ml measuring cylinder. The total volume (16.2 ml) was applied to the column as described; the measuring cylinder was washed with 2 ml picric acid which was added to the column. The total volume of the effluent and eluate was 38 ml.

2/4 Experiments

(a) Adsorption of histidine on the resin-cellulose column. Known quantities of histidine (20 to 400 µg) were dissolved in 10 ml buffer (100 mEq/l Na^+) and applied to the columns (pH 8.0); one colume was used as a control. The effluents and eluates were tested colorimetrically to measure the amount of histidine present in each fraction; the effluent and eluate from the control column were used for the blank reaction.

(b) Estimation of histamine in commercial samples of histidine. Waton (1963) has reported that samples of histidine contain variable amounts of histamine as an impurity.

Various concentrations of histidine in Tyrode's solution (50 to 800 µg/ml) were tested on the superfused guinea pig ileum (S. 2/10). Mepyramine test was also performed to exclude the presence of other active substance(s).

(c) Histidine solution exposed to various treatments involved in the procedure for extraction and purification of histamine.

(1) Two samples of histidine (50 and 100 µg in 10 ml buffer) were applied to the resin-cellul-

ose columns and the eluates were evaporated under reduced pressure and heated in 6 N HCl for 30 min at 100°C (S. 2/9).

(2) Two samples of histidine (50 and 100 μg in 5 ml water) were evaporated only.

(3) Two samples of histidine (50 and 100 μg in 5 ml water) were heated in acid.

The final dried material was reconstituted in 5 ml Tyrode's solution and assayed on the ileum for the presence of histamine activity. Similar samples of histidine solution (50 and 100 μg in 5 ml Tyrode) were used as controls.

(d) Estimation of histidine in rabbit brain and plasma after treatment with the amino acid. The procedure has already been described (Appendix 2/3). Histidine was estimated by the use of the Automatic Amino Acid Analyser. The freeze-drying and estimations were done in the Department of Therapeutics (R.I.E.).

(e) Recovery of histamine from brain tissue after the addition of known quantities of histidine. After histidine infusions (expt. d), the concentration of the amino acid in brain was about 230 μg/g (Table 46). Hence it would be expected that

10 µg of histidine would be contained in 50 mg of brain.

One cerebral hemisphere was homogenized with trichloracetic acid and water was added so that 12 mg of tissue was contained in 1 ml of extractant. Five, ten and twenty µg of histidine were added to 4 ml aliquots of the suspension and the purification procedure was carried out as described (S. 2/9). Two control samples were included.

2/5 Results

Experiment (a). Application of known quantities of histidine (dissolved in buffer: 0.05 Molar, 100 mEq/l Na^+, pH 8.0) to resin-cellulose columns and estimation of histidine colorimetrically in the effluent and eluate (Table 43).

Table 43

µg histidine added	% recovery in effluent	% recovery in eluate
20	75	undetectable
40	90	"
60	90	"
80	90,92	"
100	90	"
120	94	"
320	92	"
400	94	"
Mean	90	

The results indicate that 90% of the histidine applied to the columns passed through with the effluent and that none was detectable on elution with 0.25 N HCl, even after applying large quantities.

Histidine has 3 ionizable groups: the ionization constants for the amino and carboxylic groups at isoelectric point are 9.2 and 1.67 respectively (Levy, 1935; Deutsch and Eggleton, 1938): According to formula pK_a - pH = log Ionized/Unionized, it was calculated that at pH 8.0 (pH of the column) 94% of the amino groups and 100% of the carboxylic groups would be ionized. Hence, the net change seems to be almost nil, which might explain the lack of ion-exchange and adsorption of histidine on the resin. The 10% of unrecovered histidine may have been adsorbed non-specifically on the resin or cellulose.

Experiment (b). Histamine as contaminant in a commercial sample of histidine (Koch-Light Ltd.) (Table 44).

Table 44

Concentration of histidine base, µg/ml Tyrode	Histamine-like activity µg/g histidine
50	Undetectable
100	"
200	2.17
400	3.00
800	0.95

The stimulant effect of the solution containing 800 µg/ml Tyrode was much less than that produced by lower concentrations. This may have been due to the antihistaminic effect of histidine on the ileum. Such an effect has been described by others (Rocha e Silva, 1944; Hughes et al, 1951).

When solutions of histidine in the range 200 - 800 µg/ml, were applied to the ileum in the presence of mepyramine (3 ng/ml Tyrode's solution), there was a definite contraction which was more than that produced by 3 ng of histamine. It was concluded from these results that concentrations of 50 or 100 µg histidine/ml contained no detectable amounts of histamine. The nature of the other contaminant(s) is not known.

Experiment (c). Formation of histamine from histidine during the extraction procedure (Table 45).

Table 45

Concentration of histidine	Treatment employed	Histamine activity in the dried extract after reconstitution in Tyrode's solution
50 µg/10 ml buffer* 100 µg/10 ml buffer*	Adsorption, evaporation and heating in acid	Undetectable
50 µg/5 ml water 100 µg/5 ml water	Evaporation under reduced pressure	Undetectable
50 µg/5 ml water 100 µg/5 ml water	Evaporation and heating in acid	Undetectable
50 µg/5 ml Tyrode† 100 µg/5 ml Tyrode†	none	Undetectable

(Table 45) * 0.05 Molar, 100 mEq/2 Na^+

† Control.

The results show that when histidine was exposed to various treatments involved in the extraction and purification of histamine, no detectable amounts of histamine were being formed. These results are in accordance with those reported by Adam (1961).

Experiment (d). Effect of histidine infusions (3 x 500 mg/Kg, over 24 hr) on the concentration of the free amino acid in the rabbit brain and blood plasma (Table 46).

Table 46.

	Concentration of histidine
Blood plasma	100 µg/ml
Whole brain	230 µg/g

The concentration of histidine in plasma was found to be 5 to 10 times greater than that reported for serum histidine of untreated rabbits (10-20 µg/ml: Schwartz, Reigert and Bricka, 1938; Hughes and Williamson, 1951).

Values are not available for the concentration of free histidine in rabbit brain. But the values

reported for other species (Table 6) range from 9 to 26 μg/g. It was considered that high concentrations of histidine present in brain samples could lead to the formation of histamine during the process of extraction and purification. Hence this source of error might have accounted for some of the histamine extractable from brain.

Experiment (e). Recovery of histamine from brain tissue after the addition of known quantities of histidine (Table 47).

Table 47

Quantity of histidine added, (μg)	Histamine concentration ng/g
5	130
5	140
10	130
10	140
20	130
20	130
None	140
None	140

The concentration of histamine in brain samples to which histidine had been added was not different from that of the control, indicating that histamine was not formed during the procedure.

APPENDIX 3

EFFECT OF I:V: INJECTION OF GLYCOGEN ON THE CONCENTRATION OF HISTAMINE IN RABBIT BLOOD, HYPOPHYSIS AND BRAIN

3/1 Introduction

The object was to administer a compound which does not enter the brain but which would lower the concentration of histamine in blood. *In vitro*, glycogen releases histamine and 5-HT from rabbit platelets (Waalkes and Coburn, 1960; Westerholm, 1965). Soon after the i.v. injection of liver glycogen, the concentration of histamine in rabbit blood fell steeply; this was accompanied by a sharp fall in the platelet count; both values started to recover after 10 min and were still less than normal after 60 min (Rocha e Silva, 1950). These findings were later confirmed by Waalkes and Coburn (1959a). After the i.v. injection of glycogen (100 mg/Kg) in rabbits, the concentration of histamine and 5-HT fell in whole blood but rose in plasma and lung; the peak effects were seen after 1 min.

3/2 Materials and method

Rabbit liver glycogen (BDH) was used. A concentration of 50 mg/ml water was prepared fresh

for i.v. injection. Each rabbit received 100 mg/Kg.

The rabbit was first anaesthetized with pentobarbitone; the carotid arteries were then exposed and cannulated with polythene cannulae. Heparin was injected (800 i.u./Kg i.v.) and blood was collected from the carotid artery for haematocrit and histamine estimation. Then glycogen was injected i.v. and blood samples were collected 1, 4 and 10 min after the injection. Immediately after taking the last sample, the rabbit was bled and the brain removed and dissected (S. 2/7 and 2/8).

3/3 Results

The results (Table 48, Fig. 14) agree with those reported by Rocha e Silva (1950) and Waalkes and Coburn (1959a). The concentration of histamine in blood fell to 8% of the control 1 min. after the injection of glycogen and to 6% at 4 min; at 10 min the concentration rose to 14%. There was no evidence of change in the concentration of histamine extractable from the anterior lobe of hypophysis or brain. These findings support the conclusion that most of the histamine present in extracts of brain or hypophysis is tissue amine and not derived

Table 48

Effect of liver glycogen (100 mg/Kg i.v.) on histamine concentration in rabbit whole blood, hypophysis and brain.

Estimates of concentration as ng/g fresh tissue or ng/ml whole blood.

	Control Mean ± S.E. (No.)	after glycogen injection	
		Rabbit I	Rabbit 2
Control Blood (before injection)		5400	5400
Blood: 1 min after injection		410	480
Blood: 4 min after injection		330	340
Blood: 10 min after injection		820	680
Anterior lobe of* hypophysis	650 ± 36 (31)	530	610
Hypothalamus	660 ± 27 (25)	510	970
Central grey matter	280 ± 15 (22)	250	400
Cerebellum (vermis)	60 ± 5 (12)	40	50

No change was noted in the haematocrit reading after glycogen injection.

* Animals sacrificed at 10 min.

from blood (S. 4/3). They also confirm the view (7/3/3) that changes in brain histamine produced by treatment with reserpine are not necessarily related to the changes seen in blood.

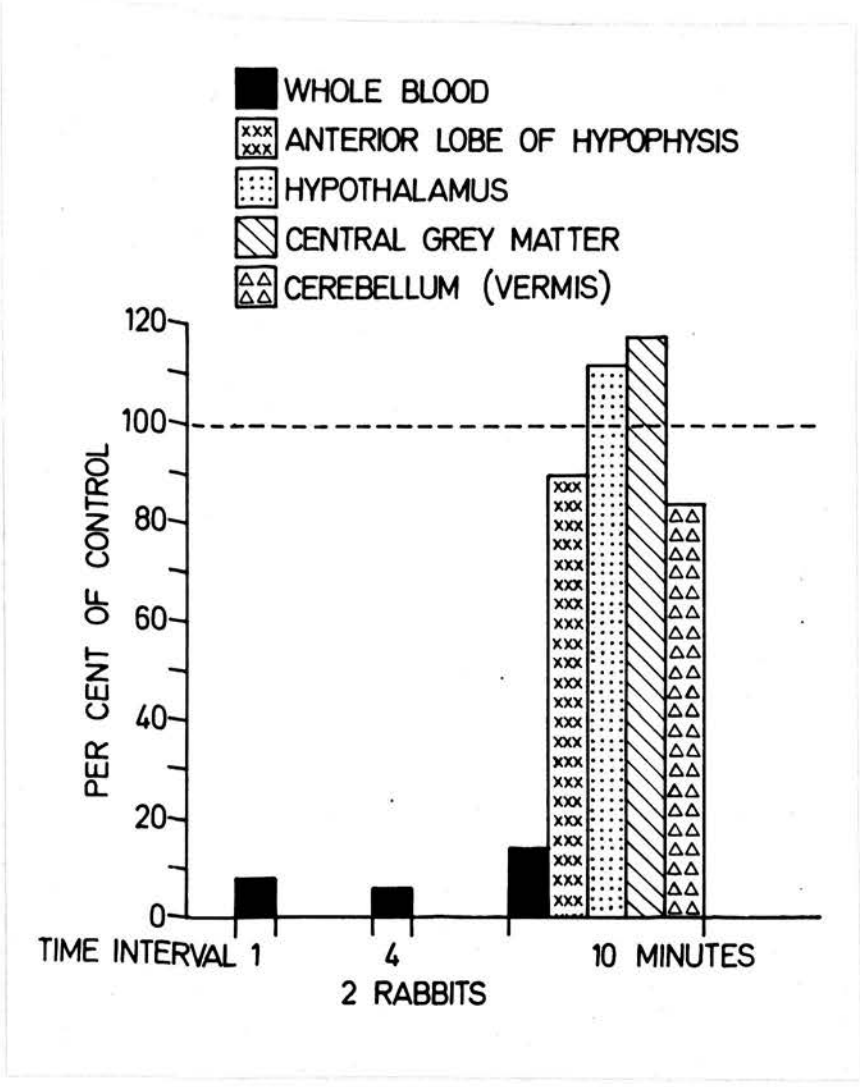


Fig. 14. Effect of i.v. injection of liver glycogen in the anaesthetized rabbit on the concentration of histamine in blood, anterior lobe of hypophysis and different regions of the brain, expressed as a percentage of the control. Blood samples were taken at 1, 4 and 10 minutes after the injection of glycogen and the concentration is expressed as a percentage of the control taken before the injection. Rabbits were sacrificed immediately after taking the last blood sample.

SUMMARY

1. Information on the occurrence and metabolism of histamine in brain, hypophysis and blood is reviewed.

2. Histamine was estimated by a method which depended on purification of the amine by ion-exchange chromatography and on biological assay.

3. A map is given of the distribution of histamine in the rabbit's brain and hypophysis. The highest concentrations were found in the hypothalamus, followed by the medial thalamus, midbrain, hind-brain, cerebral cortex and cerebellum.

4. Various experiments were performed to test for the correlation between the concentration of hist-amine in brain and that of the blood.

5. After treatment with histidine, the concentrat-ion of histamine rose in many parts of the brain and roughly paralelled the distribution of the pre-formed amine. The increase was greatest in the midbrain and hypothalmus and was dose-dependent. The disappearance of the newly-formed histamine was slow and also depended on the dose of histidine.

6. After the infusion of histamine, the concentrat-ion rose in the hypophysis but not in the hypo-thalamus or thalamus.

7. Treatment with α-methyldopa, DOPA or 5-HTP raised the concentration of histamine in the mid-brain only. Simultaneous infusion of each of these amino acids with histidine did not interfere with the formation of histamine.

8. Treatment with chlorpromazine or iproniazid raised the concentration of histamine in the mid-brain, but did not interfere with the formation of histamine from exogenous histidine.

9. After a single dose of reserpine, the concentration of histamine fell in the brain, hypophysis and blood. The effect was maximal at 16-48 hr and recovery was complete within one week.

Reserpine impaired the storage capacity of the hypothalamus for histamine whichwas marked at 24 hr but had partly recovered at 48 hr.

Reserpine released all the newly-formed histamine.

10. The results are discussed and compared with our knowledge of the distribution, formation and metabolism of biogenic amines in the brain. Possible mechanisms of the action of the amino acids and drugs are also discussed.

REFERENCES

Abdel-Latif, A.A. and Alivisatos, S.G.A. (1961). Imidazolytic processes IV. Enzymatic formation of triphosphohistamine nucleotide. J. biol. Chem. 236, 2710-2715.

Abel, J.J. and Kubota, S. (1919). On the presence of histamine (β-iminazolylethylamine) in the hypophysis cerebri and other tissues of the body and its occurrence among the hydrolytic decomposition products of proteins. J. Pharmacol. 13, 243-300

Abood, L.G. (1955). Effect of chlorpromazine on phosphorylation of brain mitochondria. Proc. Soc. exp. Biol. Med. 88, 688-690.

Abou, Y.Z., Adam, H.M. and Stephen, W.R.G. Unpublished results.

Abraham, D., Pisano, J. and Udenfriend, S. (1961). Distribution of homocarnosine in animal tissues. Fed. Proc. 20, 344.

Adam, H.M. (1961) Histamine in the central nervous system and hypophysis of the dog. In Regional Neurochemistry, ed. Kety, S.S. and Elkes, J., pp. 293-306. London, Pergamon Press.

Adam, H.M., Hardwick, D.C. and Spencer, K.E.V. (1954). Assay of histamine on the isolated guinea pig intestine by the method of superfusion. Brit. J. Pharmacol. 9, 360-366.

Adam, H.M., Hardwick, D.C. and Spencer, K.E.V. (1957). A method of estimating histamine in plasma. Brit. J. Pharmacol. 12, 397-405.

Adam, H.M. and Hye, H.K.A. (1966). Concentration of histamine in different parts of brain and hypophysis of cat and its modification by drugs. Brit. J. Pharmacol. 28, 137-152.

Adam, H.M., Hye, H.K.A. and Waton, N.G. (1964). Studies on uptake and formation of histamine by hypophysis and hypothalamus in the cat. From the Proceedings of the Physiological Society, (C. 38). Cambridge Meeting, 24-25 July. J. Physiol. 175, 70-71P.

Adam, H.M., Hye, H.K.A. and Waton, N.G. Unpublished results.

Åkerblom, E. (1941). Zur Histaminbestimmung in Gewebsextakten. Acta physiol. scand. 2, 378-380.

Albritton, E.C. (1952). Quoted in Biological Handbooks, 'Biology Data Book', ed. Altman, P.L. and Dittmer, D.S., p. 269, 1964. Federation of American Societies for Experimental Biology, Washington, DC.

Alexander, F. (1946). On the metabolism of histamine. Quart. J. exp. Physiol. 33, 71-76.

Alivisatos, S.G.A. (1958). Enzymatic interaction of histamine and coenzyme I with formation of a new dinucleotide. Fed. Proc. 17, 180.

Amin, A.H., Crawford, T.B.B. and Gaddum, J.H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol. 126, 596-618.

Anden, N. E., Roos, B. E. and Werdinius, B. (1963). 3,4-Dihydroxyphenylacetic acid in rabbit corpus striatum normally and after reserpine treatment. Life Sci. 5, 319-325.

Anden, N.E., Roos, B.E. and Werdinius, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sci. 3, 149-158.

Anrep, G.V., Barsoum, G.S., Talaat, M. and Wieninger, E. (1939). Determination of histamine in blood. J. Physiol. 95, 476-484.

Arvy, L. and Quivy, D. (1955). Données sur la répartition des labrocytes chez le chien. C.R. Ass. Anat. XLII[e] Réunion, Paris. pp. 234-241.

Ashhurst, D.E. and Richards, A.G. (1964). The histochemistry of connective tissue associated with the central nervous system of the pupa of the wax moth. J. Morphol. 114, 237-252.

Axelrod, J., MacLean, P.D., Albers, R.W. and Weissbach, H. (1961). Regional distribution of methyl transferase enzymes in the nervous system and glandular tissues. In Regional Neurochemistry, ed. Kety, S.S. and Elkes, J., pp. 307-311. New York, Pergamon Press.

Baldessarini, R.J. and Kopin, I.J. (1963). Assay of tissue levels of S-adenosylmethionine. Analyt. Biochem. 6, 289-292.

Barsoum, G.S. and Gaddum, J.H. (1935). The pharmacological estimation of adenosine and histamine in blood. J. Physiol. 85, 1-14.

Bartlet, A.L. (1960). The 5-hydroxytryptamine content of mouse brain and whole mice after treatment with some drugs affecting the central nervous system. Brit. J. Pharmacol. 15, 140-146.

Bertaccini, G. and Vitali, T. (1964). Pharmacological properties of some imidaxole derivatives occurring in nature. J. Pharm. Pharmacol. 16, 441-449.

Bertler, A. (1961). Effect of reserpine on the storage of catecholamines in brain and other tissues. Acta physiol. scand. 51, 75-83.

Bertler, Å. and Rosengren, E. (1959). On the distribution in brain of monoamines and of enzymes responsible for their formation. Experientia 15, 382-384.

Best, C:H. (1929). The disappearance of histamine from autolytic lung tissue. J. Physiol. 67, 256-263.

Best, C.H. and McHenry, E.W. (1930). The inactivation of histamine. J. Physiol. 70, 349-372.

Bhawe, W.B. (1958). Experiments on the fate of histamine and acetylcholine after their injection into the cerebral ventricles. J. Physiol. 140, 169-189.

Birkhäuser, H.V. (1940). Fermente in Gehirn geistig normaler Menschen. (Cholin-esterase, Mono-und Diamin -oxydase, Cholin-oxydase). Helv. chim. Acta 23, 1071-1086.

Blaschko, H., Friedman, P. and Nilsson, K. (1958). Two kinds of histaminase. J. Physiol. 142, 33P.

Bogdanski, D.F., Pletscher, A., Brodie, B.B. and Udenfriend S. (1956). Identification and assay of serotonin in brain. J. Pharmacol. 117, 82-88.

Bogdanski, D.F., Weissbach, H. and Udenfreind, S. (1958). Pharmacological studies with serotonin precursor, 5-hydroxytryptophan. J. Pharmacol. 122, 182-194.

Borsook, H., Deasy, C.L., Haagen-Smit, A.J., Keighley, G. and Lowy, P.H. (1950). Metabolism of ^{14}C-labelled glycine, L-histidine, L-leucine and L-lysine. J. biol. Chem. 187, 839-848.

Bovet, D., Kohn, R., Marotta, M. and Silvestrini, B. (1958). Some effects of histamine in the normal and Haemophilus pertussis vaccinated rat. Brit. J. Pharmacol. 13, 74-83.

Brandon, A. (1955). Étude électrocorticographique et électromyélographique de l'action de l'histamine. Arch. int. Physiol. 63, 62-69.

Brodie, B.B., Comer, M.S., Costa, E. and Dlabac, A. (1966). The role of brain serotonin in the mechanism of the central action of reserpine. J. Pharmacol. 152, 340-349.

Brodie, B.B., Kuntzman, R., Hirsch, C.W. and Costa, E. (1962). Effects of decarboxylase inhibition on the biosynthesis of brain monoamines. Life Sci. 3, 81-84.

Brodie, B.B., Pletscher, A. and Shore, P.A. (1956). Possible role of serotonin in brain function and in reserpine action. J. Pharmacol. 116, 9.

Brodie, B.B., Shore, P.A. and Pletscher, A. (1956). Serotonin-releasing activity limited to Rauwlfia alkaloids with tranqulizing action. Sci. 123, 992-993.

Brodie, B.B., Spector, S. and Shore, P.A. (1959). Interaction of monoamine oxidase inhibitors with physiological and biochemical mechanisms in brain. Ann. N.Y. Acad. Sci. 80 (3), 609-616.

Brown, D:D., Axelrod, J. and Tomchick, R. (1959). Enzymatic N-methylation of histamine. Nature, Lond. 183, 680.

Brown, D.D. and Kies, M.W. (1959). The mammalian metabolism of L-histidine.I. The enzymatic formation of L-hydantoin-5-propionic acid. J. biol. Chem. 234, 3182-3187.

Brown, D.D., Tomchick, R. and Axelrod, J. (1959). The distribution and properties of a histamine-methylating enzyme. J. biol. Chem. 234, 2948-2950.

Buffoni, F. (1966). Histaminase and related amine oxidases. Pharmacol. Rev. 18, 1163-1199.

Burkard, W.P., Gey, K.F, and Pletscher, A. (1963). Diamine oxidase in the brain of vertebrates. J. Neurochem. 10, 184-186.

Burkard, W.P., Gey, K.F. and Pletscher, A. (1964). Inhibition of the hydroxylation of tryptophan and phenylalanine by a-methyldopa and similar compounds. Life Sci. 3, 27-33.

Burkhalter, A., Cohn, Jr., V.H. and Shore, P.A. (1960). Effect of reserpine, serotonin, and 5-hydroxytryptophan on rabbit platelet histamine in vivo and in vitro. Biochem. Pharmacol. 3, 328-331.

Carlini, E.A., and Green, J.P. (1963). The subcellular distribution of histamine, slow-reacting substances and 5-hydroxytryptamine in the brain of the rat. Brit. J. Pharmacol. 20, 264-277.

Carlsson, A. (1966). Drugs which block the storage of 5-hydroxytryptamine and related amines. In Handbook of Experimental Pharmacology, vol. 19, '5-hydroxytryptamine and Related Indolalkylamines', ed. Eichler, O., and Farah, A., pp. 529-592. Berlin: Springer-Verlag.

Carlsson, A., and Lindqvist, M. (1962). In vivo decarboxylation of α-methyldopa and α-methylmetatyrosine. Acta physiol. scand. 54, 87-94.

Carlsson, A., Lindqvist, M., Magnusson, T., and Waldeck, B. (1958). On the presence of 3-hydroxytryptamine in brain. Sic. 127 471.

Century, B., and Horwitt, M.K. (1956). Actions of reserpine and chlorpromazine hydrochloride on rat brain oxidative phosphorylation and adenosine triphosphatase. Proc. Soc. exp. Biol. Med. 91, 493-497.

Cicardo, V.H., and Stoppani, A.O.M. (1949). Presence of histamine in central nervous system extracts. Nature, Lond. 163, 365.

Clark, C.T., Weissbach, H., and Udenfriend, S. (1954). 5-Hydroxytryptophan decarboxylase: preparation and properties. J. biol. Chem. 210, 139-148.

Clouet, D.H., Gaitonde, M.K., and Richter, D. (1957). The free histidine, histamine and arginine content of the rat brain. J. Neurochem. I, 228-233.

Code, C.F., (1937a). The quantitative estimation of histamine in the blood. J. Physiol. 89, 257-268.

Code, C.F. (1937b). The source in blood of the histamine-like constituent. J. Physiol. 90, 349-364.

Code, C.F. (1952). Histamine in blood. Physiol. Rev. 32, 47-65.

Code, C.F., Dews, P.B., and Higgins, G.M. (1953). The relationship of the adrenal gland to concentration of histamine and the number of leucocytes in the blood of rabbits. J. Physiol. 121, 487-491.

Code, C.F., and McIntire, F.C. (1956). Quantitative determination of histamine. In Methods of Biochemical Analysis, vol., 3, ed. Glick, D., pp. 49-95. New York: Interscience.

Code, C.F., and Mitchell, R.G. (1957). Histamine, eosinophils and basophils in the blood. J. Physiol. 136, 449-468.

Constantinides, P. (1953). Mast cells and susceptibility to experimental atherosclerosis. Sci. 117, 505-506.

Costa, E., and Aprison, M.H. (1958). Distribution of intracarotidly injected serotonin in the brain. Amer. J. Physiol. 192, 95-100.

Costa, E., Garattini, S., and Valzelli, L. (1960). Interactions between reserpine, chlorpromazine and imipramine. Experientia 16, 461-463.

Costa, E., Pscheidt, G.R., van Meter, W.G., and Himwich, H.E. (1960). Brain concentrations of biogenic amines and EEG patterns of rabbits. J. Pharmacol. 130, 81-88.

Costa, E., and Rinaldi, F. (1958). Biochemical and electroencephalographic changes in the brain of rabbits injected with 5-hydroxytryptophan (influence of chlorpromazine premedication). Amer. J. Physiol. 194, 214-220

Cotzias, G.C., and Dole, V.P. (1952). The activity of histaminase in tissues. J. biol. Chem. 196, 235-242.

Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P. (1953). Pharmacological properties of 3-chloro-10-(dimethylaminopropyl) phenothiazine hydrochloride. Experimental study of a new substance used in potentiating anaesthesia and artificial hibernation. Arch. int. Pharmacodyn. 92, 305-345.

Crossland, J. (1960). Chemical transmission in the central nervous system. J. Pharm. Pharmacol. 12, 1-36.

Crossland, J., and Mitchell, J.F. (1956). The effect on the electrical activity of the cerebellum of a substance present in the cerebeller extracts. J. Physiol. 132, 391-405.

Crossland, J., Mitchell, J.F., and Woodruff, G.N. (1966). The presence of ergothioñine in the central nervous system and its probable identity with the cerebeller factor. J. Physiol. 182, 427-438.

Crossland, J., Woodruff, G.N., and Woodruff, J.H. (1966). The histamine content of brain during bulbocapnine-induced catalepsy. Life Sci. 5, 193-197.

Curtis, D.R., and Davis, R. (1962). Pharmacological studies upon neurones of the lateral geniculate nucleus of the cat. Brit. J. Pharmacol. 18, 217-246.

Dale, H.H., and Laidlaw, P.P. (1911). The physiological action of β-iminazolyl-ethylamine. J. Physiol. 41, 318±344.

Darby, W.J., and Lewis, H.B., (1942). Urocanic acid and the intermediary metabolsim of histidine in the rabbit. J. biol. Chem. 146, 225-235.

Davis, R.B., Meeker, Jr., W.R., and Bailey, W.L. (1961). Serotonin release by bacterial endotoxin. Proc. soc. exp. Biol. Med. 108, 774-776.

Davison, A.N. (1958). Physiological role of monoamine oxidase. Physiol. Rev. 38, 729-747.

Davson, H. (1956). In Physiology of the Ocular and Cerebrospinal Fluid, pp. 156-163. London: J. and A. Churchill.

Day, M. and Green, J.P. (1962). The uptake of amino acids and synthesis of amines by neoplastic mast cells in culture. J. Physiol. 164, 210-226.

Decsi, L. (1961). Further studies on the metabolic background of tranquillizing drug action. Psychopharmacologia 2, 224-242.

De Jong, H.H. (1945). Experimental Catatonia. Maryland: Williams and Wilkins.

Dengler, H. and Reichel, G. (1958). Hemmung der Decarboxylase durch α-Methyldopa in vivo. Arch. exp. Path. Pharmak. 234, 275-281.

Deutsch, A. and Eggleton, P. (1938). The titration constants of anserine, carnosine and some related compounds. Biochem. J. 32, 209-211.

Documenta Geigy Scientific Tables. Ed. Diem Konrad, 6th edition.

Dragstedt, C.A. and Mead, F.B. (1935). Inactivation of histamine in vivo. Proc. Soc. exp. Biol. Med. 32, 1435-1437.

Dragstedt, C.A. and Rocha e Silva, M. (1941). Effect of trypsin upon blood histamine in rabbits. Proc. Soc. exp. Biol. Med. 47, 420-422.

Drain, D.J., Horlington, M., Lazare, R. and Poulter, G.A. (1962). The effect of α-methyldopa and some other decarboxylase inhibitors on brain 5-hydroxytryptamine. Life Sci. 3, 93-97.

Draškoci, M., Feldberg, W., Fleischhauer, K. and Haranath, P.S.R.K. (1960). Absorption of histamine into the blood stream on perfusion of the cerebral ventricles, and its uptake by brain tissue. J. Physiol. 150, 50-66.

Edlbacher, S. (1943). Histidase and urocaninase. Ergebnisse der Enzymforschung 9, 131-154.

Ewins, A.J. and Pyman, F.L. (1911). Experiments on the formation of 4 (or 5) -β-amino-ethylglyoxaline from histidine. J. chem. Soc. 99, 339-344.

Emmelin, N. (1945). Ultrafiltration as a method of preparing blood plasma for quantitative estimation of histamine. Acta physiol. scand. 9, 378-381.

Emmelin, N. (1951). Disappearance of injected histamine from the blood stream. Acta physiol. scand. 22, 379-390.

Emmelin, N., Kahlson, G. and Wicksell, F. (1941). Histamine in plasma and methods for its estimation. Acta physiol. scand. 2, 123-142.

Emmerson, J.L. and Miya, T.S. (1963). Metabolism of phenothiazine drugs. J. Pharmaceut. Sci. 52, 411-419.

Erspamer, V. (1954). Pharmacology of indolealkylamines. Pharmacol. Rev. 6, 425-487.

Erspamer, V. (1961). Pharmacologically active substances of mammalian origin. Ann. Rev. Pharmacol. 1, 175-218.

Euler, U.S.v. (1950). Histamine as a specific constituent of certain autonomic nerve fibres. Acta physiol. scand. 19, 85-93.

Euler, U.S.v. (1958). The presence of adrenergic neurotransmitter in intra-axonal structures. Acta physiol. scand. 43, 155-166.

Feldberg, W. and Greengård, P. (1956). Release of histamine from the perfused sciatic nerve by 48/80. J. Physiol. 133, 63P-64P.

Feldberg, W. and Kwiatkowski, H. (1933). Untersuchungen über primären und sekundären Histaminschock am arteriellen Blutdruck. Arch. exp. Path. Pharmak. 170, 551-559.

Feldberg, W. and Sherwood, S.L. (1954). Injections of drugs into the lateral ventricle of the cat. J. Physiol. 123, 148-167.

Feldberg, W. and Smith, A.N. (1953). Release of histamine by tryptamine and 5-hydroxytryptamine. Brit. J. Pharmacol. 8, 406-411.

Finkleman, B. (1930). On the nature of inhibition of the intestine. J. Physiol. 70, 145-157.

Fram, D.H. and Green, J.P. (1963). The presence of methylhistamine in the brain. Pharmacologist 5, 253.

Fram, D.H. and Green, J.P. (1965). Methylhistamine in urine and brain. Fed. Proc. 24, 778.

Friedberg, F. and Greenberg, D.M. (1947). Partition of intravenously administered amino acids in blood and tissues. J. biol. Chem. 168, 411-413.

Gaddum, J.H. (1953). The technique of superfusion. Brit. J. Pharmacol. 8, 321-326.

Gaddum, J.H. (1956). The origin of histamine in the body. In Ciba Foundation Symposium, 'Histamine', ed. Wolstenholme, G.E.W. and O'Connor, C.M., pp. 285-297. London: Churchill.

Gaddum, J.H. and Giarman, N.J. (1956). Preliminary studies on the biosynthesis of 5-hydroxytryptamine. Brit. J. Pharmacol. 11, 88-92.

Gamble, H.J. (1964). Comparative electron-microscopic observations on the connective tissue of a peripheral nerve and a spinal nerve root in the rat. J. Anat., Lond. 98, 17-25.

Gamble, H.J. and Goldby, S. (1961). Mast cells in peripheral nerve trunks. Nature, Lond. 189, 766-767.

Ganrot, P.O., Rosengren, A.M. and Rosengren, E. (1961). On the presence of different histidine decarboxylating enzymes in mammalian tissues. Experientia 17, 263-264.

Gertner, S.B. and Kohn, R. (1959). The effect of histamine on ganglionic transmission. Brit. J. Pharmacol. 14, 179-182.

Gey, K.F. and Pletscher, A. (1961). Influence of chlorpromazine and chlorprothixine on the cerebral metabolism of 5-hydroxytryptamine, norepinephrine and dopamine. J. Pharmacol. 133, 18-24.

Gilfoil, T.M., Hart, E.R. and Marrazzi, A.S. (1960). Cerebral synaptic inhibition by histamine. Fed. Proc. 19, 262.

Goldstein, L., Pfeiffer, C.C. and Munoz, C. (1963). Quantitative EEG analysis of the stimulant properties of histamine and histamine derivatives. Fed. Proc. 22, 424.

Graham, H.T., Lowry, O.H., Wheelwright, F., Lenz, M.A. and Parish, H.H. (1955). Distribution of histamine among leucocytes and platelets. Blood 10, 467-481.

Graña, A. and Rocha e Silva, M. (1945). Effect of hydatid fluid on histamine content of rabbit blood. Amer. J. Physiol. 143, 314-323.

Gray, J.H. (1935). Preliminary note on the mast cells of human pituitary and of the mammalian pituitary in general. J. Anat., Lond. 69, 153-158.

Green, H. and Erickson, R.W. (1964). Effect of some drugs upon rat brain histamine content. Int. J. Neuropharmacol. 3, 315-320.

Green, J.P. (1962). Binding of some biogenic amines in tissues. Advanc. Pharmacol. 1, 349-423.

Green, J.P. (1964). Histamine and the nervous system. Fed. Proc. 23, 1095-1102.

Green, J.P. (1967). Uptake and binding of histamine. Fed. Proc. 26, 211-218.

Green, J.P. and Furano, A.V. (1962). Two pools for amines in neoplastic mast cells. Biochem. Pharmacol. 11, 1049-1053.

Guggenheim, M. and Löeffler, u.W. (1916). Biologischer Nachweis Proteinogener Amine in Organextracten und Körperflüssigkeiten. Biochem. Z. 72, 303-324.

Gursey, D. and Olson, R.E. (1960). Depression of serotonin and norepinephrine levels in brain stem of rabbit by ethanol. Proc. Soc. exp. Biol. Med. 104, 280-281.

Gustafsson, A. and Forshell, G.P. (1963). Purification of N-methyl transferase. Acta chem. scand. 17, 541-542.

Hagen, P., Barnett, R.J. and Lee, F.L. (1959). Biochemical and electron microscopic study of particles isolated from mastocytoma. J. Pharmacol. 126, 91-108.

Hagen, B.P. and Cohen, L.H. (1966). Biosynthesis of indolealkylamines. Physiological release and transport of 5-hydroxytryptamine. In Handbook of Experimental Pharmacology, vol. 19, '5-Hydroxytryptamine and Related Indolealkylamines', ed. Eichler, O. and Farah, A., pp. 182-211. Berlin: Springer-Verlag.

Hagen, P., Weiner, N., Ono, S. and Lee, F.L. (1960). Amino acid decarboxylase of mouse mastocytoma tissue. J. Pharmacol. 130, 9-12.

Halpern, B.N., Neveu, T. and Wilson, C.W.M. (1959). The distribution and fate of radioactive histamine in the rat. J. Physiol. 147, 437-449.

Handbook of Biological Data (1956). ed. Spector, W.S. London: Saunders.

Handbook of Toxicology (1959). Vol. IV, 'Tranquillizers', ed. Grebe, R.M. London: Saunders.

Harris, G.W., Jacobsohn, D. and Kahlson, G. (1952). The occurrence of histamine in cerebral regions related to the hypophysis. In Ciba Foundation Colloquia on Endocrinology, vol. 4, pp. 186-194. London: Churchill.

Heath, R.G., Leach, B.E. and Verster, F. de B. (1962). In Hallucinations, ed. West, L.J., p. 183. New York: Grune and Stratton.

Hebb, C.O. and Whittaker, V.P. (1958). Intracellular distribution of acetylcholine and choline acetylase. J. Physiol. 142, 187-196.

Hess, S.M., Connamacher, R.H., Ozaki, M. and Udenfriend, S. (1961). The effects of α-methyldopa and α-methyl-meta-tyrosine on the metabolism of norepinephrine and serotonin in vivo. J. Pharmacol. 134, 129-138.

Hess, S.M. and Doepfner, W. (1961). Behavioural effects and brain amine content in rats. Arch. int. Pharmacodyn. 134, 89-99.

Hess, S.M., Redfield, B.G. and Udenfriend, S. (1959). The effect of monoamine oxidase inhibitors and tryptophan on the tryptamine content of animal tissues and urine. J. Pharmacol. 127, 178-181.

Hess, S.M., Shore, P.A. and Brodie, B.B. (1956). Persistence of reserpine action after disappearance of drug from brain: effect on serotonin. J. Pharmacol. 118, 84-89.

Himwich, H.E., Costa, E., Pscheidt, G.R. and Van Meter, W.G. (1959). Discussion. Ann. N.Y. Acad. Sci. 80(3), 614-616.

Holmes, F. and Jones, F. (1960). Metal complexes of histamine and some structural analogues. Part I. J. chem. Soc. 2, 2398-2401.

Holtz, P. and Heise, R. (1937). Über Histaminbildung in Organismus. Arch. exp. Path. Pharmak. 186, 377-386.

Holtz, P. and Westermann, E. (1956). Über die dopa-decarboxylase und histidindecarboxylase des Nervengewebes. Arch. exp. Path. Pharmak. 227, 538-546.

Horita, A. and Gogerty, J.H. (1958). The pyrotogenic effect of 5-hydroxytryptophan and its comparison with LSD. J. Pharmacol. 122, 195-200.

Hughes, D.E., Salvin, E. and Wood, D.R. (1951). Decarboxylation of histidine as a source of error in extracting histamine from tissues. J. Physiol. 113, 218-227.

Hughes, D.E. and Williamson, D.H. (1951). Removal of acid by trioctylamine from samples for microbiological assay. Biochem. J. 48, 487-490.

Humphrey, J.H. and Jaques, R. (1954). The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species. J. Physiol. 124, 305-310.

Hye, H.K.A. (1964). Ph.D. Thesis, University of Edinburgh. "On the concentration of histamine in different parts of the brain and hypophysis of the cat and its modification by drugs."

Johnson, H.L., Beaven, M.A., Erjavec, F. and Brodie, B.B. (1966). Selective labeling and release of nonmast-cell histamine. Life Sci. 5, 115-123.

Jonson, B. and White, T. (1964). Histamine metabolism in the brain of conscious cats. Proc. Soc. exp. Biol. Med. 115, 874-876.

Joyce, D. (1962). Changes in the 5-hydroxytryptamine content of rat, rabbit and human brain after death. Brit. J. Pharmacol. 18, 370-380.

Kahlson, G. and Rosengren, E. (1965). Histamine. Ann. Rev. Pharmacol. 5, 305-320.

Kahlson, G., Rosengren, E., Westling, H. and White, T. (1958). The site of increased formation of histamine in pregnant rat. J. Physiol. 144, 337-348.

Kamin, H. and Handler, P. (1951). The metabolism of parenterally administered amino acids. J. biol. Chem. 188, 193-205.

Kapeller-Adler, R. and Renwick, R. (1956). On the enzymatic breakdown of histamine and cadaverine in human serum and urine. Clin. chim. Acta 1, 197-209.

Karjala, S.A. (1955). The partial characterization of a histamine metabolite from rat and mouse urine. J. Amer. chem. Soc. 77, 504-505.

Kärki, N.T., Paasonen, M.K. and Peltola, P. (1960). Factors influencing the release of 5-hydroxytryptamine from the rabbit intestine into the venous blood. Ann. Med. exp. Fenn. 38, 231-236.

Katoka, K. and De Robertis, E. (1967). Histamine in isolated small nerve endings and synaptic vesicles of the rat brain cortex. J. Pharmacol. 156, 114-125.

Kelsall, M.A. and Lewis, P. (1964). Mast cells in the brain. Fed. Proc. 23, 1107-1108.

Kiyokawa, M. (1933). Beiträgezur Kenntnis der biologischen Spaltung des histidins. Z. physiol. Chem. 214, 38-41.

Kobayashi, Y. and Schayer, R.W. (1956). In vitro demonstration of a new histamine-metabolizing enzyme. Arch. Biochem. Biophys. 63, 480-481.

Koechlin, B. and Iliev, V. (1959). Metabolism of iproniazid. Ann. N.Y. Acad. Sci. 80 (3), 864-872.

Koessler, K.K. and Hanke, M.T. (1919). Studies on proteinogenous amines. II. A microchemical colorimetric method for estimating imidazole derivatives. J. biol. Chem. 39, 497-519.

Kolb, E. (1956). Histaminase and diamine oxidase in the serum of domestic animals (ruminant, pig, horse) and in Escherichia coli and Streptococcus faecalis. Zbl. Vet. Med. 3, 570-591.

Kremzner, L.T. (1965a). Determination of brain histamine by fluorometric assay. Fed. Proc. 24 (2), 578.

Kremzner, L.T. (1965b). Method for the determination of histamine. Fed. Proc. 24 (1), 518.

Kremzner, L.T. and Pfeiffer, C.C. (1966). Identification of substances interfering with the fluorometric determination of brain histamine. Biochem. Pharmacol. 15, 197-200.

Kremzner, L.T. and Wilson, I.B. (1961). A procedure for the determination of histamine. Biochim. biophys. Acta 50, 364-367.

Krnjevic, K. and Phillis, J.W. (1963). Actions of certain amines on cerebral cortical neurones. Brit. J. Pharmacol. 20, 471-490.

Kunz, E. (1964). Vergleichende Wirkung von zwei Decarboxylasehemmern auf den Metabolismus von endogenem und exogenem 5-Hydroxytryptamain. Arch. int. Pharmacodyn. 147, 1-8.

Kwiatkowski, H. (1941). Observations on the relation of histamine to reactive hyperaemia. J. Physiol. 100, 147-158.

Kwiatkowski, H. (1943). Histamine in nervous tissue. J. Physiol. 102, 32-41.

Lagunoff, D. and Benditt, E.P. (1963). Proteolytic enzymes of mast cells. Ann. N.Y. Acad. Sci. 103 (1), 185-198.

Lajtha, A. and Mela, P. (1961). The Brain Barrier System - I. The exchange of free amino acids between plasma and brain. J. Neurochem. 7, 210-217.

Lee, H.M. and Jones, R.G. (1949). The histamine activity of some β-aminoethyl heterocyclic nitrogen compounds. J. Pharmacol. 95, 71-78.

Leroy, J.G. (1961). 5-Hydroxytryptamine levels in brain of mice pretreated with α-methyldopa. Arch. int. Pharmacodyn. 134, 492-496.

Levine, R.J., Sato, T.L. and Sjoerdsma, A. (1965). Inhibition of histamine synthesis in the rat by α-hydrazino analog of histidine and 4-bromo-3-hydroxybenzyloxyamine. Biochem. Pharmacol. 14, 139-149.

Levine, R.J. and Sjoerdsma, A. (1964). Dissociation of the decarboxylase-inhibiting and norepinephrine-depleting effects of α-methyldopa, α-ethyl-dopa, 4-bromo-3-hydroxy-benxyloxyamine and related substances. J. Pharmacol. 146, 42-47.

Levy, M. (1935). A note on the titration constants of imidazole derivatives. J. biol. Chem. 109, 361-363.

Lewis, B., Gebauer-Fuelnegg, E.v. and Farmer, C.J. (1933). A spectroscopic study and assay of histamine. J. Amer. chem. Soc. 55 (2), 2025-2027.

Lewis, T. (1927). The Blood Vessels of the Human Skin and Their Responses. London: Shaw.

Lewis, T. and Grant, R.T. (1924). Vascular reactions of the skin to injury. II. The liberation of a histamine-like substance in injured skin: the underlying cause of factitious urticaria and of wheals produced by burning; and observations upon the nervous control of certain skin reactions. Heart 11, 209-265.

Lin, T.M. (1961). Work done at Lilly Research Laboratories, unpublished. In Handbook of Experimental Pharmacology, vol. 18/1, 'Histamine and Anti-histamines'; ed. Eichler, O. and Farah, A., pp. 1-43. Berlin: Springer-Verlag.

Lindahl, K.M. (1958a). Methylation of histamine by mouse liver in vitro. Acta physiol. scand. 43, 254-261.

Lindahl, K.M. (1958b). On the biological methylation of histamine. Acta chem. scand. 12 (2), 1690-1691.

Lindahl, K.M. (1958c). On the biological methylation of histamine. 3. Acta chem. scand. 12 (2), 2050-2051.

Lindahl, K.M. (1960). The histamine methylating enzyme system in liver. Acta physiol. scand. 49, 114-138.

Lindell, S.E., Nilsson, K., Roos, B.-E. and Westling, H. (1960). The effect of enzyme inhibition on histamine catabolism in man. Brit. J. Pharmacol. 15, 351-355.

Lindell, S.-E. and Viske, K. (1961). A note on the distribution of ^{14}C-histamine added to blood. Brit. J. Pharmacol. 17, 131-136.

Lindell, S.E. and Westling, H. (1957). Enzymatic oxidation of some substances related to histamine. Acta physiol. scand. 39, 370-384.

Lovenberg, W., Weissbach, H. and Udenfriend, S. (1962). Aromatic L-amino acid decarboxylase. J. biol. Chem. 237, 89-93.

Mackay, D., Riley, J.F. and Shepherd, D.M. (1961). Amino-acid decarboxylase activities in rat hepatoma. J. Pharm. Pharmacol. 13, 257-261.

Mackay, D. and Shepherd, D.M. (1960). A study of potential histidine decarboxylase inhibitors. Brit. J. Pharmacol. 15, 552-556.

Macpherson, H.T. (1946). The basic amino-acid content of proteins. Biochem. J. 40, 470-481.

McGeer, E.G., McGeer, P.L. and McLennan, H. (1961). The inhibitory action of 3-hydroxytryptamine, gamma-aminobutyric acid (GABA) and some other compounds towards the crayfish stretch receptor neuron. J. Neurochem. 8, 36-49.

McGeer, P.L. (1964). The distribution of histamine in cat and human brain. In Comparative Neurochemistry, ed. Richter, D., pp. 387-391. London: Pergamon Press.

McIntire, F.C., White, F.B. and Sproull, M. (1950). The determination of histamine with 2,4-Dinitrofluorobenzene. Arch. Biochem. Biophys. 29, 376-386.

Magee, W.L., Berry, J.F. and Rossiter, R.J. (1956). Effect of chlorpromazine and azacylonol on the labelling of phosphatides in brain slices. Biochim. biophys. Acta 21, 408-409.

Markwardt, F., Barthel, W. and Glusa, E. (1966). Die Veränderungen des Serotonin- und Histamingehaltes der Blutplättchen unter dem Einfluss von Lokalanaesthetica. Arch. exp. Path. Pharmak. 253, 336-344.

Matsuoka, M., Yoshida, H. and Imaizumi, R. (1964). Distribution of catecholamines and their catabolites in rabbit brain. Biochim. biophys. Acta 82, 439-441.

Michaelson, A. (1967). Spermidine: a contaminant in the n-butanol extraction of brain in the fluorometric assay of histamine. Europ. J. Pharmacol. 1, 378-382.

Michaelson, I.A. and Coffman, P.Z. (1967). The association of histamine with nerve endings in the dog hypothalamus. Pharmacologist 9, 348.

Michaelson, I.A. and Dowe, G. (1963). The subcellular distribution of histamine in brain tissue. Biochem. Pharmacol. 12, 949-956.

Michaelson, I.A. and Whittaker, V.P. (1963). The subcellular localization of 5-hydroxytryptamine in guinea pig brain. Biochem. Pharmacol. 12, 203-211.

Minard, D. (1937). The presence and distribution of histamine-like substance in blood. Amer. J. Physiol. 119, 375.

Minard, D. (1941). The presence and distribution of histamine in blood. Amer. J. Physiol. 132, 327-335.

Mongar, J.L. and Schild, H.O. (1950). Apparatus for pharmacological assay. J. Physiol. 111, 47P.

Monnier, M. (1960). Actions électro-physiologiques des stimulants du système nerveus central. I. Système adrénergiques, cholinergiques et neurohumeurs sérotoniques. Arch. int. Pharmacodyn. 124, 281-301.

Monnier, M. and Tissot, R. (1958). Action de la Réserpine et de ses Mediateurs (5-hydroxytryptophan-Sérotonine et Dopa-Noradrénaline) sur le comportement e le serveau du lapin. Helv. physiol. pharmacol. Acta 16, 255-267.

Murphy, G.F. and Sourkes, T.L. (1959). Effect of catecholamino acids on the catecholamine content of rat organs. Rev. canad. Biol. 18, 379-388.

Murphy, G.F. and Sourkes, T.L. (1961). The action of antidecarboxylases on the conversion of 3,4-dihydroxyphenylalanine to dopamine in vivo. Arch. Biochem. Biophys. 93, 338-343.

Neame, K.D. (1961). Uptake of amino acids by mouse brain slices. J. Neurochem. 6, 358-366.

Neame, K.D. (1962). Uptake of L-histidine, L-proline, L-tyrosine, L-ornithine by brain, intestinal mucosa, testes, kidney, spleen, liver, heart muscle, skeletal muscle and erythrocytes of the rat in vitro. J. Physiol. 162, 1-12.

Neame, K.D. (1964). Uptake of histidine, histamine and other imidazole derivatives by brain slices. J. Neurochem. 11, 655-662.

Nepryakhin, G.G. (1960). On mast cells in the nervous system. Archiv Patologii 22 (10), 53-59.

Oehme, V.C. (1913). Über die Wirkungsweise des Histamins. Archiv. exp. Path. Pharmak. 72, 76-96.

Padawer, J. (1957). Studies on mammalian mast cells. Trans. N.Y. Acad. Sci. 19 (8), 690-713.

Parkes, M.W. (1961). Tranquillizers. In Progress in Medical Chemistry, vol. 1, ed. Ellis, G.P., pp. 72-131. London: Butterworths.

Parrat, J.R. and West, G.B. (1957). Release of 5-hydroxytryptamine and histamine from tissues of the rat. J. Physiol. 137, 179-192.

Perry, T.L., Hansen, S., Foulks, J.G. and Ling, G.M. (1965). Aliphatic and aromatic amines of cat brain. J. Neurochem. 12, 397-405.

Perry, T.L., Hansen, S. and Jenkins, L.C. (1964). Amine content of the normal human cerebrospinal fluid. J. Neurochem. 11, 49-53.

Pilcher, J.D. and Sollmann, T. (1914). Studies in the vasomotor centre. XII. The action of ergot and its constituents, ergotoxin, and histamine. J. Pharmacol. 6, 385-389.

Pisano, J., Abraham, D. and Udenfriend, S. (1963). Gamma-Aminobutyrlhistidine in brain. J. Neuropsych. 4, 264-265.

Pletscher, A. (1963). Drug-induced alterations of monoamine metabolism. In The Clinical Chemistry of Monoamines, ed. Varley, H. and Gowenlock, A.H., pp. 191-204. London: Elsevier.

Pletscher, A., Gey, K.F. and Burkard, W.P. (1966). In Handbook of Experimental Pharmacology, vol. 19, '5-Hydroxytryptamine and Related Indolealkylamines', ed. Eichler, O. and Farah, A., pp. 613-618. Berlin: Springer-Verlag.

Pletscher, A., Shore, P.A. and Brodie, B.B. (1956). Serotonin as a mediator of reserpine action in brain. J. Pharmacol. 116, 84-89.

Pyman, F.L. (1911). Aminoalkylglyoxalines. J. chem. Soc. 99, 2172-2183.

Quensel, U. (1928). Some investigations concerning mast cells. Acta path. microbiol. scand. Suppl. 5, 34-37.

Reid, J.D. and Shepherd, D.M. (1963). Inhibition of histidine decarboxylase. Life Sci. 1, 5-8.

Riesser, O. (1937). Histaminstudien. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmakol. 187, 1-21.

Riley, J.F. and West, G.B. (1955). Tissue mast cells. Studies with a histamine liberator of low toxicity (compound 48/80). J. Path. Bact. 69, 269-282.

Robinson, J.D., Anderson, J.H. and Green, J.P. (1965). The uptake of 5-hydroxytryptamine and histamine by particulate fractions of brain. J. Pharmacol. 147, 236-243.

Robinson, J.D. and Green, J.P. (1964). Presence of imidazoleacetic acid riboside and ribotide in rat tissues. Nature, Lond. 203, 1178-1179.

Robinson, J.D. and Green, J.P. (1965). Evidence for the presence of imidazoleacetic acid riboside and ribotide in the rat tissues. Fed. Proc. 24, 777.

Rocha e Silva, M. (1944). Inhibition of histamine effects by compounds of histamine, histidine and arginine. J. Pharmacol. 80, 399-408.

Rocha e Silva, M. (1950). The role played by leucocytes and platelets in anaphylactic and peptone shock. Ann. N.Y. Acad. Sci. 50, 1045-1067.

Rocha e Silva, M. and Essex, H.E. (1942). Effect of animal poisons (rattlesnake venom and trypsin) on blood histamine of guinea pigs and rabbits. Amer. J. Physiol. 135, 372-377.

Rocha e Silva, M., Graña, A. and Porto, A. (1945). Inhibitory effect of glycogen upon anaphylactic shock in the rabbit. Proc. Soc. exp. Biol. Med. 59, 57-61.

Roos, B.-E. and Werdinius, B. (1962). Effect of reserpine on the level of 5-hydroxyindoleacetic acid in brain. Life Sci. 3, 105-107.

Roos, B.-E. and Werdinius, B. (1963). The effect of α-methyldopa on the metabolism of 5-hydroxytryptamine in brain. Life Sci. 2, 92-96.

Rose, B. (1941). Studies on the histamine-content of the blood and tissues of the rabbit during anaphylactic shock. J. Immunol. 42, 161-180.

Rose, B. and Browne, J.S.L. (1938). The distribution and rate of disappearance of intravenously injected histamine in the rat. Amer. J. Physiol. 124, 412-420.

Rose, B. and Weil, P. (1939). Blood histamine in the rabbit during anaphylactic shock. Proc. Soc. exp. Biol. Med. 42, 494-496.

Rosengren, E. (1960). Are dihydroxyphenylalanine decarboxylase and 5-hydroxytryptophan decarboxylase individual enzymes? Acta physiol. scand. 49, 364-369.

Rosenthal, S.M. and Tabor, H. (1948). An improved colorimetric method for the estimation of histamine. J. Pharmacol. 92, 425-431.

Rothschild, Z. and Schayer, R.W. (1958). Synthesis and metabolism of a histamine metabolite, 1-methyl-4-(β-aminoethyl)-imidazole. Biochim. biophys. Acta 30, 23-27.

Rothschild, A.M. and Schayer, R.W. (1959). Characterization of histidine decarboxylase from rat peritoneal fluid mast cells. Biochim. biophys. Acta 34, 392-398.

Sankar, S.D.V., Phipps, E., Gold, E. and Sankar, D.B. (1962). Effect of LSD, BOL and chlorpromazine on "Neurohormone" metabolism. Ann. N.Y. Acad. Sci. 96 (1), 93-97.

Schayer, R.W. (1952). Biogenesis of histamine. J. biol. Chem. 199, 245-250.

Schayer, R.W. (1956a). Formation and binding of histamine by free mast cells of rat peritoneal fluid. Amer. J. Physiol. 186, 199-202.

Schayer, R.W. (1956b). Formation and binding of histamine by rat tissues in vitro. Amer. J. Physiol. 187, 63-65.

Schayer, R.W. (1959). Catabolism of physiological quantities of histamine in vivo. Physiol. Rev. 39, 116-126.

Schayer, R.W. (1962). Evidence that induced histamine is an intrinsic regulator of microcirculatory system. Amer. J. Physiol. 202, 66-72.

Schayer, R.W. (1966). Catabolism of histamine in vivo. In Handbook of Experimental Pharmacology, vol. 18/1, 'Histamine and Anti-histamines', ed. Eichler, O. and Farah, A., pp. 672-683. Berlin: Springer-Verlag.

Schayer, R.W. and Karjala, S.A. (1956). Ring-N-methylation, a major route of histamine metabolism. J. biol. Chem. 221, 307-313.

Schayer, R.W. and Kobayashi, Y. (1956). Histidine decarboxylase and histamine binding in rabbit platelets. Proc. Soc. exp. Biol. Med. 92, 653-655.

Schayer, R.W. and Sestokas, E. (1965). Suppression of histamine synthesis in guinea pigs by α-methyl-3, 4-dihydroxyphenylalanine. Biochim. biophys. Acta 111, 557-558.

Schild, H.O. (1947). The use of drug antagonists for the identification and classification of drugs. Brit. J. Pharmacol. 2, 251-258.

Schmiterlow, C.G. (1950). Some remarks on the determination of histamine with special reference to horse blood. Acta physiol. scand. 19, 260-269.

Schurr, P.E., Thompson, H.T., Henderson, L.M., Williams, J.N., Jr. and Elvehjem, C.A. (1950). The determination of free amino acids in rat tissues. J. biol. Chem. 182, 39-45.

Schwartz, A., Reigert, A. and Bricka, M. (1938). Contribution au microdosage des imidazols, en particulier de l'histidine, dans le serum sanguin. C.R. Soc. Biol., Paris, 129, 1159-1162.

Shore, P.A. (1962). Release of serotonin and catecholamines by drugs. Pharmacol. Rev. 14, 531-550.

Shore, P.A. and Brodie, B.B. (1957). Influence of various drugs on serotonin and norepinephrine in brain. In Psychotropic Drugs, ed. Garattini, S. and Ghetti, V., pp. 423-427. Amsterdam: Elsevier.

Shore, P.A., Burkhalter, A. and Cohn, V.H. Jr. (1959). A method for the fluorometric assay of histamine in tissues. J. Pharmacol. 127, 182-186.

Shore, P.A., Pletscher, A., Tomich, E.G., Carlsson, A., Kuntzman, R. and Brodie, B.B. (1957). Role of brain serotonin in reserpine action. Ann. N.Y. Acad. Sci. 66 (3), 609-617.

Shore, P.A., Pletscher, A., Tomich, E.G., Kuntzman, R. and Brodie, B.B. (1956). Release of blood platelet serotonin by reserpine and lack of effect on bleeding time. J. Pharmacol. 117, 232-236.

Smith, S.E. (1960). The pharmacological actions of 3,4-dihydroxyphenyl-α-methylalanine (α-methyldopa), an inhibitor of 5-hydroxytryptophan decarboxylase. Brit. J. Pharmacol. 15, 319-327.

Snyder, S.H. and Axelrod, J. (1964). Inhibition of histamine methylation in vivo by drugs. Biochem. Pharmacol. 13, 536-537.

Snyder, S.H. and Axelrod, J. (1965). Tissue metabolism of ^{14}C-histamine *in vivo*. Fed. Proc. 24, 774-776.

Snyder, S.H., Axelrod, J. and Bauer, H. (1964). The fate of ^{14}C-histamine in animal tissues. J. Pharmacol. 144, 373-379.

Snyder, S.H., Baldessarini, R.J. and Axelrod, J. (1966). A sensitive and specific enzymatic isotopic assay for tissue histamine. J. Pharmacol. 153, 544-549.

Snyder, S.H., Glowinski, J. and Axelrod, J. (1966). The physiologic disposition of ^{3}H-histamine in the rat brain. J. Pharmacol. 153, 8-14.

Sokoloff, L. (1959). The action of drugs on the cerebral circulation. Pharmacol. Rev. 11, 1-85.

Sokoloff, L. (1961). Local cerebral circulation at rest and during altered cerebral activity induced by anaesthesia or visual stimulation. In Regional Neurochemistry, ed. Kety, S.S. and Elkes, J., pp. 107-117. London: Pergamon Press.

Sourkes, T.L. (1954). Inhibition of dihydroxyphenylalanine decarboxylase by derivatives of phenylalanine. Arch. Biochem. Biophys. 51, 444-456.

Sourkes, T.L. (1965). The action of α-methyldopa in the brain. Brit. Med. Bull. 21, 66-69.

Sourkes, T.L. and Murphy, G.F. (1960). Effects of catecholamino acids on catecholamine (CA) content of rat organs. Fed. Proc. 19, 295.

Spector, S. (1963). Monoamine oxidase in control of brain serotonin and norepinephrine content. Ann. N.Y. Acad. Sci. 107 (3), 856-864.

Spector, S., Kuntzman, R., Shore, P.A. and Brodie, B.B. (1960). Evidence for release of brain amines by reserpine in presence of monoamine oxidase inhibitors: implication of monoamine oxidase in norepinephrine metabolism. J. Pharmacol. 130, 256-261.

Spector, S., Shore, P.A. and Brodie, B.B. (1960). Biochemical and pharmacological effects of monoamine oxidase inhibitors, iproniazid, 1-phenyl-2-hydrazino-propane (JB 835). J. Pharmacol. 128, 15-21.

Strengers, T. and Maas, J.W. (1956). Studies of histamine IV. On free and bound histamine. Acta allerg. 10, 130-135.

Szeberényi, S. and Kovács, K. (1959). Über den Einfluss endokriner Factoren auf den Histamingehalt des Hypothalamus der Ratte. Naturwissenschaften 46, 581-582.

Tabor, H. (1951). Diamine oxidase. J. biol. Chem. 188, 125-136.

Tabor, H. (1954). Metabolic studies on histidine, histamine and related imidazoles. Pharmacol. Rev. 6, 299-343.

Tabor, H. and Hayaishi, O. (1955). The excretion of imidazoleacetic acid riboside following the administration of imidazoleacetic acid or histamine to rats. J. Amer. chem. Soc. 77, 505-506.

Tallan, H.H., Moore, S. and Stein, W.H. (1954). Studies on the free amino acids and related compounds in the tissues of the cat. J. biol. Chem. 211, 927-939.

Tallan, H.H., Stein, W.H. and Moore, S. (1954). 3-Methylhistidine, a new amino acid from human urine. J. biol. Chem. 206, 825-834.

Tarras-Wahlberg, B. (1937). Über den Histamingehalt der Haut nach Ultravioletbestrahlung. Klin. Wschr. 16(2), 958-960.

Trendelenburg, U. (1957a). Stimulation of sympathetic centres by histamine. Circulation Res. 5, 105-110.

Trendelnburg, U. (1957b). The action of histamine, pilocarpine and 5-hydroxytryptamine on transmission through the superior cervical ganglion. J. Physiol. 135, 66-72.

Tsusaki, V.T., Eriguchi, K. and Kojo, Y. (1951). Über die basophil granulierten zellen im plexus choroideus. Yokohama Med. Bull. 2, 110-117.

Udenfriend, S. (1958). In 5-Hydroxytryptamine, ed., Lewis, G.P., p. 48. London: Pergamon Press.

Udenfriend, S., Lovenberg, W.M. and Weissbach, H. (1960). L-Amino acid decarboxylase activity in mammalian tissues and its inhibition by α-methyldopa. Fed. Proc. 19, 7.

Udenfriend, S. and Weissbach, H. (1958). Turnover of 5-hydroxytryptamine (serotonin) in tissues. Proc. Soc. exp. Biol. Med., 97, 748-751.

Udenfriend, S., Weissbach, H., and Bogdanski, D.F. (1957). Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan. J. biol. Chem. 224, 803-810.

Ungar, G. and Witten, J.W. (1963). Increase in brain histamine caused by tremorine. Fed. Proc. 22, 273.

Valentine, W.N., Lawrence, J.S., Pearce, M.L. and Beck, W.S. (1955). The relationship of the basophil to blood histamine in man. Blood 10, 154-159.

Vartiainen, A. (1935). The action of certain new histamine derivatives. J. Pharmacol. 54, 265-282.

Virno, M., Gertner, S.B. and Bovet, D. (1956). Action of histamine on the jugular venous pressure and cerebral circulation of the dog. Effects of antihistaminic drugs (pyrilamine and chlorpheniramine) and a histamine liberating agent (48/80 B.W.). J. Pharmacol. 118, 63-76.

Vogt, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123, 451-481.

Vogt, M. (1959). Catecholamines in brain. Pharmacol. Rev. 11, 483-489.

Waalkes, T.P. and Coburn, H. (1959a). Comparative effects of glycogen and antigen-antibody reactions on serotonin and histamine in the rabbit. Proc. Soc. exp. Biol. Med. 101, 122-125.

Waalkes, T.P. and Coburn, H. (1959b). The role of platelets and the release of serotonin and histamine during anaphylaxis in the rabbit. J. Allergy 30, 394-407.

Waalkes, T.P. and Coburn, H. (1960). Studies on the relationship of the release of serotonin and histamine, by chemical means, to anaphylaxis in the rabbit. J. Allergy 31, 395-405.

Waalkes, T.P., Coburn, H. and Terry, L.L. (1959). The effect of reserpine on histamine and serotonin. J. Allergy 30, 408-414.

Waalkes, T.P. and Weissbach, H. (1956). In vivo release of histamine from rabbit blood by reserpine. Proc. Soc. exp. Biol. Med. 93, 394-396.

Walaszek, E.J. and Chapman, J.E. (1963). Abstracts of papers: 2nd International Pharmacological Meeting, Prague 20-23 Aug. Addendum. Biochem. Pharmacol. 12, 36.

Waldeyer, W. (1875). Uber Bindegewebszellen. Arch. mikr. Anat. 11, 176-194.

Wase, A.W., Christensen, J. and Polley, E.H. (1956). Accumulation of ^{35}S-chlorpromazine in brain. Arch. Neurol. Psychiat., Chicago. 75, 54-56.

Waton, N.G. (1956). Studies on mammalian histidine decarboxylase. Brit. J. Pharmacol. 11, 119-127.

Waton, N.G. (1963). Histamine as an impurity in samples of histidine. J. Pharm. Pharmacol. 15, 574-578.

Weil-Malherbe, H., Posner, H.S. and Waldrop, F.N. (1962). The alleged effect of schizophrenic serum on rabbit brain catecholamines. Ann. N.Y. Acad. Sci. 96(1), 419-424.

Weissbach, H., Bogdanski, D.F. and Udenfriend, S. (1958). Binding of serotonin and other amines by blood platelets. Arch. Biochem. Biophys. 73, 492-499.

Weissbach, H., Lovenberg, W.M., Udenfriend, S. (1961). Characteristics of mammalian histidine decarboxylating enzymes. Biochem. biophys. Acta. 50, 177-179.

Weissbach, H. and Redfield, B.G. (1960). Factors affecting the uptake of 5-hydroxytryptamine in human platelets in an inorganic medium. J. biol. Chem. 235, 3287-3291.

Weissbach, H., Waalkes, T.P. and Udenfriend, S. (1957). Presence of serotonin in lung and its implication in the anaphylactic reaction. Sci. 125, 235-236.

Weissbach, H., Waalkes, T.P. and Udenfriend, S. (1958). A simple method for measuring serotonin in tissues; simultaneous assay of both serotonin and histamine. J. biol. Chem. 230, 865-871.

Werle, E. (1936). Über die Bildung von Histamin aus Histidin durch tierisches Gewebe. Biochem. Z. 288, 292-293.

Werle, E. (1961). Hemmung der Histidin-Decarboxylase durch α-Methyldopa. Naturwissenschaften 48, 54-55.

Werle, E. and Aures, D. (1959). Über die Reiningung und Spezifität der DOPA- Decarboxylase. Z. physiol. Chem. 316, 45-60.

Werle, E. and Herrmann, U.H. (1937). Über die Bildung von Histamin aus Histidin durch tierisches Gewebe. Biochem. Z. 191, 105-121.

Werle, E. and Krautzun, K. (1938). Über die Bildung von Histamin aus Histidin durch tierische Gewebe. II. Biochem. Z. 296, 315-324.

Werle, E. and Palm, D. (1950). Histamin in Nerven, II. Biochem. Z. 320, 322-334.

Werle, E. and Palm, D. (1952). Histamin in Nerven, III. Biochem. Z. 323, 255-264.

Werle, E. and Schauer, A. (1956). Histamin in Nerven. Z. ges. exp. Med. 127, 16-21.

Werle, E. and Weicken, G. (1949). Über das Vorkmmen von Histamin in Nerven. Biochem. Z. 319, 457-462.

Weschsler, M.B. and Forrest, I.S. (1959). A quantitative method for the determination of chlorpromazine in tissues. J. Neurochem. 4, 366-371.

West, G.B. (1957). Histamine in the nervous system. In Metabolism of the Nervous System, ed. Richter, D., pp. 578-581. London: Pergamon Press.

Westerholm, B. (1965). Observations on 5-hydroxytryptamine and histamine release from rabbit platelets. Acta. physiol. scand. 63, 257-270.

Westermann, E., Blazer, H. and Knell, J. (1958). Hemmung der serotoninbildung durch α-methyldopa. Arch. exp. Path. Pharmak. 234, 194-205.

White, T. (1959). Formation and catabolism of histamine in brain tissues in vitro. J. Physiol. 149, 34-42.

White, T. (1960). Formation and catabolism of histamine in cat brain in vivo. J. Physiol. 152, 299-308.

White, T. (1961a). Inhibition of methylation of histamine in cat brain. J. Physiol. 159, 191-197.

White, T. (1961b). Some effects of histamine and two histamine metabolites on cat's brain. J. Physiol. 159, 198-202.

White, T. (1966). Histamine and methylhistamine in cat brain and other tissues. Brit. J. Pharmacol. 26, 494-501.

Whittaker, V.P. (1959). The isolation and characterization of acetylcholine containing particles from brain. Biochem. J. 72, 694-706.

Williams, J.N.Jr., Schurr, P.E. and Elvehjem, C.A. (1950). The influence of chilling and exercise on free amino acid concentration in rat tissue. J. biol. Chem. 182, 55-59.

Wilson, A. (1941). The effect of adrenalectomy on the blood histamine of rabbits. J. Physiol. 99, 241-245.

Wislocki, G.B. and Leduc, E.H. (1952). Vital staining of hematoencephalic barrier by sliver nitrate and trypan blue, and cytological comparisons of neurohypophysis, pineal body, area postrema, intercolumnar tubercle and supraoptic crest. J. Comp. Neurol. 96, 371-413.

Yuwiler, A., Geller, E. and Eiduson, S. (1959). Studies on 5-hydroxytryptophan decarboxylase. I. In vitro inhibition and substrate interaction. Arch. Biochem. Biophys. 80, 162-173.

Zeller, E.A. (1938). Über den enzymatischen Abbau von Histamin und Diaminen. Helv. Chim. Acta. 21. 880-890.

Zeller, E.A. (1951). Diamine oxidase. In The Enzymes II, ed. Summer, J.B. and Myrbäck, K., pp. 544-558. New York: Academic Press.

Zeller, E.A. (1965). Identity of histaminase and diamine oxidase. Fed. Proc. 24, 766-768.

Zeller, E.A., Birkhäuser, H., Mislin, H. and Wenk, M. (1939). Über das Vorkmmen der Diamin-oxydase bei Mensch, Säugtier und Vogel. Miteinem Anhang über das Vorkmmen der Cholin-esterase beim Vogel. Helv. chim. Acta 22, 1381-1393.

Zeller, E.A., Stern, P. and Blanksma, L.A. (1956). Degradation of histamine by monoamine oxidase. Naturwissenschaften 43, 157.

Zimmermann, A. (1908). Über das Vorkmmen der Mastzellen beim Meerschweinchen. Arch. mikr. Anat. scand. 62, 662-669.

Zon, L.E., Ceder, E.T., and Crigler, C.W. (1939). The presence of histamine in the platelets of the rabbit. U.S. Pub. Hlth. Rep. 54(2), 1978-1986.